ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, $4.00 PER YEAR

JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXV WASHINGTON, D. C., AUGUST 18, 1923 No. 7

QUANTITATIVE VARIATION OF GOSSYPOL AND ITS RELATION TO THE OIL CONTENT OF COTTONSEED[1]

By ERICH W. SCHWARTZE, *Pharmacologist in Charge, Pharmacological Laboratory,* and CARL L. ALSBERG, *formerly Chief, Bureau of Chemistry, United States Department of Agriculture*

INTRODUCTION

In connection with the food conservation problem which arose during the World War, the Bureau of Chemistry was asked to supply information on the suitability of cottonseed press cake for human consumption.[2] As the statements in the literature on the nature of poisoning by cottonseed were conflicting, a reinvestigation of the question was necessary. The investigation resolved itself into several distinct, though closely related, studies. While all of these studies were carried on simultaneously, each one will be reported separately, beginning with the one here discussed.[3]

For a long time some feeders have believed that different lots of cottonseed meal have different degrees of toxicity.[4, 5] It is highly probable that there is a sound basis for this belief on the part of practical men. Crawford (*6*), who stated that the toxic agent in cottonseed is pyrophosphoric acid, regarded some of these differences, at least, as varietal characteristics of the different seed. This would be consistent with the belief very generally held that cottonseed poisoning is more frequent in some regions than in others, since the varieties cultivated differ from region to region. At any rate, it seems to be a fact that a variation in toxicity is associated with variations in the place and crop year of production, although the writers have been unable to find in the literature satisfactory data on which a comparison of poisoning by cottonseed produced in different regions or from the crops of different years in the same region could be based.

That the composition of many seeds varies from region to region and from crop year to crop year has been established beyond doubt. In the case of wheat these variations are common knowledge. Piper and Morse (*8*) have observed that certain regions of the South produce soybeans with higher oil content than others. Thompson and Bailey (*11*) found that differences in the oil content of different varieties of peanuts grown under the same conditions and in the same locality were not

[1] Accepted for publication June 23, 1923. This is the first paper of a series dealing with cottonseed poisoning.

[2] Bryan (*1*) recommends cottonseed meal, in limited quantities, for human consumption. He does not discuss, however, the deleterious action of this meal on domesticated animals.

[3] The preliminary report on this work was read before the American Chemical Society, April 7–11, 1919. See ALSBERG, C. L., SCHWARTZE, E. W., and WHERRY, E. T. THE OCCURRENCE OF GOSSYPOL IN DIFFERENT VARIETIES OF COTTONSEED. (Title.) *In* Science, v. 49, p. 573. 1919.)

[4] Reference is made by number (italic) to "Literature cited," p. 295.

[5] Personal communication from C. T. Dowell, Stillwater, Okla.

pronounced. Similar facts have been established for cottonseed by Bidwell,[6] who has shown that cottonseed from the southwestern United States, on the average, is low in oil and high in protein, while seed from the Atlantic and Pacific coasts has a higher oil and lower protein content in the order named. While Bidwell's generalization is undoubtedly justified, exceptions must be anticipated, for over such large territories climatic and soil conditions must be exceptional here and there. This being so, exceptions will be encountered, if any reliance can be placed upon the work of Bain and Anders as reported by Cook (*5*), who writes as follows:

> The fluctuations induced by conditions of growth or associated with various degrees of maturity attained by the seeds were so large as to conceal inherent differences in individual plants or progenies.

Hence the finding by Rast (*9*) of great variations in composition of Georgia cottonseed is not astonishing nor to be regarded as invalidating Bidwell's generalization.

If it be true that cottonseed varies greatly in composition and toxicity in different parts of the country and perhaps also in different crop years, then the toxic factor in cottonseed, whatever it may be, might well vary correspondingly. If Withers and Carruth (*12, 13*) are right in attributing the toxicity of cottonseed to the presence in it of a phenolic substance named by Carruth "gossypol," then the gossypol content of different samples of cottonseed should vary as the toxicity varies. Apparently Carruth (*3*), recognizing that gossypol occurs in the so-called "gland dots" or "resin glands" in cottonseed, the distribution of which has been studied by Stanford and Viehoever (*10*), does not believe the gossypol content of cottonseed to vary greatly. He states that "since all varieties of seed seem to have approximately the same number of glands it would appear that gossypol does not vary to a greater extent than the oil or protein content." This statement, however, is not based on experimental evidence. Hence it is just as reasonable to assume that, even if the number of glands in different cottonseeds were the same, the glands might vary both in size and in gossypol content. Carruth (*3*) found gossypol in cotton-root bark, where, according to the histological studies of Stanford and Viehoever (*10*), there are also internal glands. Carruth (*3*) also attributed the differences of toxicity to variations in the method of manufacture of the press cake. However, this suggestion, while it may apply in some cases, does not explain the infrequency of poisoning in certain regions where the method of manufacture of the meal is generally the same as that in regions where poisoning is prevalent.

There is, then, very definite evidence that the composition of cottonseed varies widely and no evidence inconsistent with the possibility that the gossypol content may vary correspondingly. To determine whether or not the gossypol content of cottonseed varies and to determine whether there is any correlation between the gossypol, the protein, and the oil content is the purpose of the present paper. A study of the oil and protein content was included in the investigation, so that three criteria instead of one might be available for checking the results. The protein and oil analyses could be used to determine whether or not the sample was representative of the region from which the seed came and also to rule out "sports" or atypical seed. Consideration of the oil and protein content made it unnecessary to consider in detail the various factors affecting the seed.

[6] Personal communication from G. L. Bidwell, Bureau of Chemistry.

This study, then, deals solely with the maximum variation of the gossypol content of cottonseed and the correlation of the gossypol content with the oil and protein content of the seed. No consideration is given to the biological factors that might cause the gossypol content to vary. No consideration is given to methods of selection, breeding, or cultivation that might lead to the production of seed of low gossypol content.

SOURCES OF SEED EXAMINED

Samples of most of the standard varieties and some of the same variety from different localities were secured. Most of the seed examined was obtained through the offices of O. F. Cook and R. A. Oakley, of the Bureau of Plant Industry, United States Department of Agriculture, which maintains an inspection of plantations on which its seeds are grown. Therefore, there can be no doubt as to the authenticity of the varieties of the seed furnished. A few samples were obtained directly from planters, experiment stations, or dealers.

METHODS OF EXAMINATION

Only the kernels or meats of the seeds were examined. Usually a very small quantity of hull and lint, which could not be removed from the ground material by sifting, was present. Gossypol was isolated as the "acetate" by the methods of Carruth (*3*) from all varieties of seed used. All samples of gossypol "acetate" isolated were examined crystallographically. Quantitative analysis was made according to the writers' modification of the aniline method of Carruth (*4*). The fat and moisture determinations upon the samples of material analyzed for gossypol were made in the Cattle Food Laboratory, and the nitrogen determinations by the Kjeldahl method were made in the Nitrogen Section of the Bureau of Chemistry.

MODIFIED ANILINE METHOD

Place 75 gm. of practically hull-free finely ground cottonseed meats in a Soxhlet extraction thimble. Extract with ether until the thimble which stands in the ether overnight imparts to it no significant yellow color. Evaporate the ether completely and transfer the extract to a beaker, using petroleum ether to work it over with. If necessary, filter it. Use 8 to 10 times as much petroleum ether as the volume of the extract.

After standing overnight, a very small quantity of a fine flocculent precipitate appears. This is not gossypol, for while gossypol is not soluble in petroleum ether alone, it remains in the oil-petroleum-ether mixture. Filter off this petroleum-ether-insoluble material extracted by the ether and wash the precipitate with petroleum ether. Wash the precipitate and the filter paper with ether. Filter this ether solution and evaporate almost completely. Mix the residuum with petroleum ether in order to hold in solution the last traces of oil and petroleum-ether-soluble material. Then filter and combine the filtrate with the main petroleum-ether solution. Add 1 cc. of aniline and dissolve it in the solution by shaking. Unless dissolved, the gossypolaniline compound comes out in clusters around the small drops of aniline.

In from 3 to 7 days later filter the precipitate of aniline-gossypol compound through a tared Gooch crucible and wash it several times with

petroleum ether. Rub off the material adhering to the precipitating flask with a rubber-tipped glass rod or dissolve it in ether, from which it may be precipitated by almost completely evaporating the ether, adding petroleum ether, and reevaporating to a small volume. Then pour it carefully into the Gooch crucible. Bring the Gooch crucible and its contents to constant weight at 100° C. by hourly heatings. This precipitate is slightly hygroscopic. Carry on the heating no longer than is necessary. If filter paper has been used in the Gooch crucible, the precipitate can be removed without admixture of foreign substances.

Preserve the filtrate containing the petroleum-ether-aniline mixture and transfer it to an Erlenmeyer flask. Stopper the flask and let it stand in the cold for from seven days to one month, to ascertain whether all the gossypol has come down. If gossypol appears, allow the petroleum ether to evaporate partly and let stand again. Corrections based on these subsequent precipitates may be made.

APPLICATION OF MODIFIED ANILINE METHOD

Table I shows the gossypol content of several varieties of cottonseed determined by the aniline method of Carruth (*4*) and also by the qualitative "acetate" method (*3*), in which care was taken to make the yields as large as possible. These data show that the recoveries by the "acetate" method are from 16 to 33 per cent lower than those by the aniline method. The approximate agreement of the results obtained by the two procedures lends weight to the assumption that the aniline method gives data representing the gossypol content.

TABLE I.—*Gossypol obtained by the "acetate" and aniline methods*

Variety of cottonseed.	Acetate method.[a]	Aniline method.[b]
	Per cent.	*Per cent.*
Lone Star	0.27	[illegible]
Acala	.324	[illegible]
Trice	.401	[illegible]
Durango	0.636	[illegible]
Egyptian	0.610	[illegible]
Sea Island	1.018	[illegible]

[a] The acetic acid in gossypol "acetate" is approximately 10 per cent and has been deducted.
[b] A Kjeldahl analysis was made and the nitrogen, calculated as aniline, was deducted from the weight of the precipitate.
[c] Some was lost.

The nitrogen content of the aniline precipitate was from 3.75 to 4.05 per cent. The variation was due, to some extent at least, to the adsorption of free aniline. A lower nitrogen content has been observed and a preparation free from the odor of aniline has been obtained upon recrystallizing several times from chloroform. Usually more nitrogen was present in the more bulky precipitates from the Gooch crucible, owing probably to less favorable conditions for removal of aniline in the heating. The variability in aniline content of the aniline-gossypol compound was noted by Carruth also (*3*). The weight of gossypol was calculated by deducting the weight of the aniline ($N \times 6.64$) from the weight of the precipitate. This correction of the deduction, however, could perhaps

be dispensed with by using the general average, as the increased accuracy obtained by considering variations in nitrogen content is within the limits of error of the method.

The use of petroleum ether as a medium for precipitation facilitated the formation of a more filterable mass, and expedited the separation of the aniline compound. It also accelerated the rate of filtration. During the ether extraction some material other than fat or gossypol was dissolved. This was removed from the petroleum-ether mixture before the aniline was added.

When the crude ether extracts of cottonseed kernels or the mother liquors obtained in the process of recrystallization of gossypol were treated with aniline, the precipitate formed had a dull red color. When this precipitate was recrystallized from chloroform, a few crystals which differed from aniline-gossypol and which could be separated mechanically were usually obtained. When purified gossypol was converted into the aniline compound, crystals of this second type were not obtained. Although the second substance was not studied in detail, it seems probable that it is the aniline compound of the "D-gossypol" of Carruth. The aniline method precipitates not only gossypol, but also gossypollike substances, the quantity of which, however, was relatively small.

Carruth states that the error of his method is less than 10 per cent when 0.5 gm. of gossypol is dissolved in 50 cc. of oil. The results (Table I) by his method, however, are somewhat lower than those subsequently obtained with the same seed by the authors' modification of his procedure. The results of the control analyses for the estimation of known quantities of free gossypol are given in Table II.

TABLE II.—*Estimation of known quantities of gossypol*

Weight of free gossypol taken.	Dissolved in 25 cc. of—	Total weight of precipitates.[d]	Aniline contained in precipitates.[e]	Gossypol recovered.	
Gm.		*Gm.*	*Per cent.*	*Gm.*	*Per cent.*
[a] 0.7002	Peanut oil	0.8736	26.07	0.6459	92.24
[a] 0.6642	do	.8424	25.77	.6253	94.14
[b] 0.6902	Cottonseed oil [c]	.8296	26.50	.6097	88.34
[b] 0.6902	do	.8534	27.10	.6221	90.13
[b] 0.6902	do	.8594	26.67	.6302	91.37
[b] 0.6902	do	.8562	26.67	.6279	90.97

[a] Dried in desiccator over calcium chlorid.
[b] On moisture-free basis, 0.7000 gm. actually taken.
[c] Refined in Oil, Fat, and Wax Laboratory of the Bureau of Chemistry.
[d] Including weight of all subsequent small precipitates.
[e] Calculated on basis of nitrogen analysis.

The error would appear to be on the average about 10 per cent of 0.7 gm. of free gossypol dissolved in 25 cc. of cottonseed oil, equivalent to about 3 mgm. per cubic centimeter of evaporated ether extract. The seeds showing a low gossypol content are not equally poor in oil. There is, therefore, a greater percentage of error in determining the gossypol in the samples of seed running low in gossypol.

A gram sample of gossypol "acetate" gave no weighable ash. Kjeldahl analysis of two preparations of "free" gossypol gave 0.0363 and 0.033 per cent of nitrogen. This small quantity is not significant and undoubtedly represents an impurity in the preparations.

IDENTIFICATION OF GOSSYPOL

Gossypol was identified as the "acetate" in all varieties of cotton-seed (Table III) which were analyzed quantitatively, also in Wine Sap (a red-foliage variety), in several samples of gin-run seed, and in Ingenhouzia (Arizona wild cotton). In addition, it was secured from a sample of commercial cotton-root bark. These preparations of gossypol are described as follows, by Dr. Edgar T. Wherry, crystallographer of the Bureau of Chemistry, who found the optical properties to be identical with those of the preparations which were submitted to the writers by Doctor Carruth:

OPTICAL-CRYSTALLOGRAPHIC PROPERTIES OF GOSSYPOL "ACETATE"

All of the samples of gossypol submitted proved to be crystalline, practically insoluble in the usual organic immersion liquids and well adapted for optical-crystallographic study under the microscope. Their properties are as follows:

IN ORDINARY LIGHT.—Consists of bright yellow flakes, often rather acutely rhombic in outline, or sometimes approximately hexagonal. Two or more acute crystals are sometimes grown together to form a twin, with a deep reentrant angle at one end. The crystal system is apparently triclinic.

WITH POLARIZING NICOL.—Pleochroism is very slight, but pseudo-absorption is marked.

REFRACTIVE INDICES.—(20°/D): alpha = 1.530 to 1.540, beta = 1.750 to 1.760, gamma = 1.820 to 1.830, gamma − alpha = 0.290. The grains usually lie in positions oblique to the index directions, so that mean values are shown. By working over large masses of crystal fragments however, the individual indices can be obtained without great difficulty. The evidence for variation in indices from one preparation to another is definite. This variation, however, is not associated with any other recognizable difference in crystallography, and appears to be due to variation in amount of solvent-of-crystallization or perhaps of material present in solid solution in the crystals.

IN PARALLEL POLARIZED LIGHT, NICOLS CROSSED.—The birefringence is extreme and interference colors are shown only by the thinnest flakes, rarely reaching low orders. Extinction is inclined, the angle varying widely with the orientation of the crystals, but being 20° toward certain frequently occurring edges. Elongation is often negative, but likewise varies with the orientation.

IN CONVERGENT POLARIZED LIGHT, NICOLS CROSSED.—Partial bi-axial interference figures are not difficult to obtain. The axial angle $2E$ is large, probably around 100°. The sign is negative, and dispersion is extremely strong and markedly unsymmetrical.

COMPOSITION OF COTTONSEED

The results of the gossypol determinations, together with those for fat, nitrogen, and moisture content, are given in Table III.

The data in Table III show that the gossypol content of seed may vary by as much as 300 per cent. (Compare Trice, 1918, and Lone Star, 1918, with the Egyptian and Columbia, 1918.) The greatest annual variation in any one variety, approximately 200 per cent, was observed in Trice

seed from Bells, Tenn. (Compare seed of 1917, 1918, and 1919.) Smaller annual variations were noted in certain other seeds, and practically none in others. The authors' series, however, is rather limited, and it is possible that other large annual variations occur frequently in other varieties, particularly in regions which have variable or occasionally unfavorable weather conditions.

TABLE III.—*Gossypol, oil, protein, and moisture content of cottonseed meats*

Variety.	Place grown.	Year grown.	Moisture.	Ether extract.	Nitrogen.	Gossypol found.		
			Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
Lone Star	Greenville, Tex	1917 or 1918	5.93	31.46	6.19	0.4137	0.3833	0.3869
Do[1]	Yuma Valley, Bard, Calif.[2]	1919	5.64	33.06	5.88	.4636	.4649	.....
Do	Greenville, Tex	1919(?)	5.65	33.97	6.30	.5307	.5084	.....
Do[3]	do	1919	.....	33.40	6.40	.5288	.5076	.....
Do[4]	Texas	1919	5.42	34.72	5.87	[6].6322	[6].6401	.....
Do[4]	Arkansas	1919	5.84	35.30	5.84	[5].6849	[5].6770	.....
Do	Manchester, N. C	1919	5.66	35.45	5.62	.6592	.6899	.....
Do[5]	Courtland, Ala	1919	5.11	38.28	5.45	.7392	.7410	.....
Do[5]	do	1919	6.04	37.13	5.20	.7970	.7982	.....
Do[6]	Bakersfield, Calif	1919	5.68	35.92	5.60	.8581	.8761	.....
Do	Elizabeth City, N. C	1919	5.82	38.46	4.91	.9076	.....	.....
Durango[6]	Courtland, Ala	1919	5.88	37.16	5.27	.7176	.6970	.....
Do	Bakersfield, Calif	1919	5.66	37.02	4.82	.8574	.8709	.....
Do	Columbia, S. C	1918	5.79	36.66	5.83	.8236	.9072	.....
Do[6]	Yuma Valley, Bard, Calif.	1919	5.45	37.33	5.13	.8846	.9312	.8831
Do[7]	do.[2]	1919	5.69	37.40	4.77	.9552	.9319	.....
Do[6]	Georgia, North Carolina, South Carolina.	1919	5.38	38.87	5.19	.9811	.9849	.....
Do[3]	Columbia, S. C	1918(?)	5.93	38.97	4.94	.9843	.9833	.....
Do	Columbia, S. C. (?)	1917 or 1918	4.81	40.60	4.88	1.0603	1.0348	.....
Trice[3]	Bells, Tenn	1918	6.12	28.87	6.42	.3970	.4250	.....
Do	do	1917	5.45	32.51	6.28	.5776	.5797	.....
Do	do	1919	6.53	33.84	5.75	.8893	.9061	.....
Do[4]	Tennessee	1919	.....	.....	.....	.9426	.9524	.....
Do[4]	Eastern States	1919	.....	.....	.....	[5] 1.0592	[5] 1.1812	.....
Kasch	Clarksville, Tex	1918(?)	5.44	33.69	6.04	.4574	.4560	.....
Do	do	1919	6.21	34.56	5.71	.5554	.5557	.5778
Do[4]	Oklahoma	1919	4.92	35.41	5.70	.8976	[5].9094	[6].9067
Do[6]	Bakersfield, Calif	1919	5.25	40.98	4.40	.9639	.9512	.....
Meade	Charleston, S. C	1918 or 1919	5.52	37.95	4.97	.5856	.5742	.....
Do	Ware County, Ga	1918	5.06	37.87	5.08	.6455	.6446	.....
Dixie[4]	Eastern States	1919	5.72	38.27	5.00	[5] 1.0185	.....	.....
Do	Florence, S. C	1919	5.74	39.52	4.66	1.0305	1.0366	.....
Columbia	Easley, S. C	1918	5.46	38.89	4.85	1.1105	1.1162	.....
Do[4]	South Carolina	1919	.....	.....	.....	[5] 1.0064	[5] 1.0278	.....
Egyptian[3]	Sacatone, Ariz	1918	5.66	36.68	4.73	1.1832	1.1758	.....
Do[6]	Bakersfield, Calif	1919	5.59	36.08	5.34	1.1745	1.1847	.....
Kanz[3]	Richmond, Va	1919(?)	5.24	38.35	5.14	.9219	.9185	.....
Cleveland[3]	St. Matthews, S. C	1919(?)	6.49	35.20	5.25	.7127	.6892	.....
Sea Island[8]	Blackshear, Ga	1918	.....	.....	.....	[5].9446	.....	.....

[1] Probably the first year's growth from seeds imported into this region.
[2] Received from the agricultural experiment station.
[3] Used for feeding tests.
[4] The Bureau of Plant Industry distributes seed and receives from planters samples of their trial crops for examination. These seeds were composite samples from a wide range of territory.
[5] Estimated on the basis of 75 per cent gossypol in aniline gossypol.
[6] Received from planter.
[7] Received from seed dealer. Seed probably from immediately adjacent territory.

Table III also shows that the same variety grown in widely separated regions may or may not contain different quantities of gossypol. This indicates that influences other than those of a varietal character play a significant rôle. In direct agreement with this deduction is the observation that different varieties grown in the same region contained approximately the same quantity of gossypol. One exception was Lone Star from Bard, Calif., which was much lower in gossypol than Durango from

the same place. Although not typical of the region from which it came, this sample of Lone Star is in itself not atypical of cottonseed. Egyptian seed from Bakersfield, Calif., contained a slightly larger quantity of gossypol than the three other varieties from this place. This, as well as the high gossypol content of Egyptian seed obtained from Arizona, suggests that there may be slight differences between *Gossypium herbaceum* and *Gossypium hirsutum*, although the single analysis of Sea Island seed does not confirm this.

The results of all the analyses indicate that the occurrence of an intoxication due to gossypol would not be influenced by the variety of seed from which the meal is made, but rather by the place from which it came and the season in which the seed is grown. If a varietal influence upon the gossypol content actually exists, practically it is concealed. The manner of cultivation (agronometric) also probably plays a rôle.

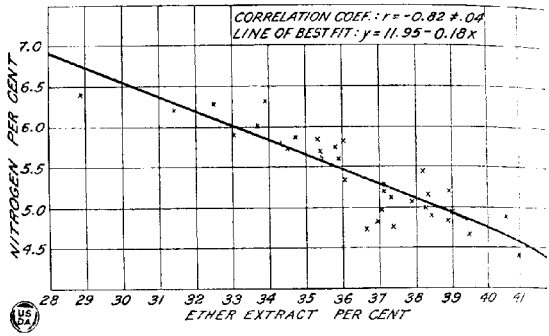


FIG. 1.—Relation between ether extract and nitrogen content of cottonseed. Since r is negative, the nitrogen content decreases as the ether extract content increases. The value of r (0.82), which is large as compared with its probable error (0.04), is significant. The partial correlation coefficient (0.01) indicates a significant relationship between the ether extract and nitrogen content.

Although only presumptive evidence upon this point exists in these experiments, it is a logical supposition to make from the results of Bain and Anders reported by Cook (5).

Figures 1, 2, and 3 give the mathematical expression and interpretation of the writer's data.[7] In these computations each individual gossypol analysis has been used. The correlation coefficients [8] between ether extract (oil) and nitrogen (protein), and between gossypol and ether extract, respectively, show that relationships exist, but that they are not perfect. The reality of these relationships is further borne out by the determination of partial correlation coefficients.

An apparent correlation exists between the nitrogen (protein) and the gossypol; in fact, the results of one analysis may be used to a certain extent to estimate the other. That this relationship may be false is shown by the fact that their partial correlation coefficient is very low

[7] The calculations were made and the charts were plotted by J. C. Munch.

[8] The correlation coefficient is a measure of the relationship between two variables. It is a relative value, zero, (0) indicating no relationship and unity (1) perfect relationship. The partial correlation coefficient indicates the relationship between two variables when other known variables are eliminated.

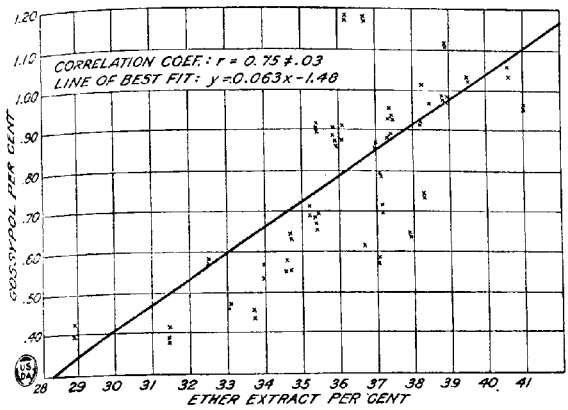


FIG. 2.—Relation between ether extract and gossypol content of cottonseed. Since r is positive, the gossypol content increases as the ether extract content increases. The value for r (0.75), which is large as compared with its probable error (0.03), is significant. The partial correlation coefficient (0.45) indicates a significant relationship between the ether extract and gossypol content.

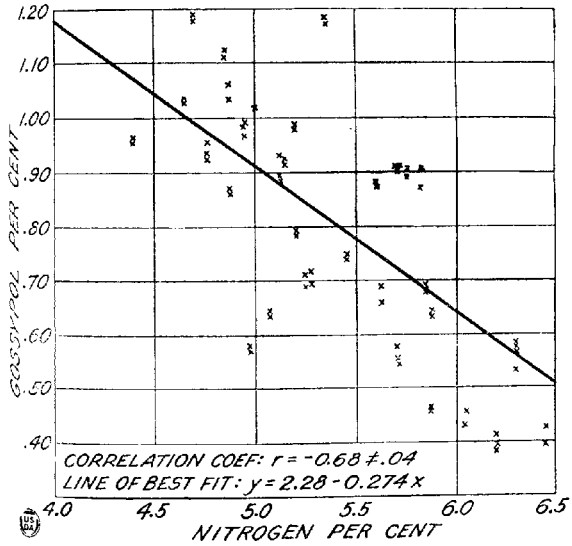


FIG. 3.—Relation between nitrogen and gossypol content of cottonseed. Since r is negative, the gossypol content decreases as the nitrogen content increases. The value for r (0.68) which is large as compared with its probable error (0.04), is significant. The partial correlation coefficient (0.17) indicates, however, that the correlation may be false.

It is probably true that this apparent relation is due to the close relationship of each of these to the oil.

Before basing generalizations on the gossypol content of cottonseed upon these analyses, it is to be noted that the nitrogen (protein) and the oil content of the seeds fall fairly well within the generalization of Bidwell concerning the interrelation of the quantities of these two constituents in seeds from different parts of the Cotton Belt. This conformity indicates that the small number of samples of seeds analyzed form a representative series. The seeds from the Southwest have a tendency to be low in oil, those from the Southeast to be somewhat higher, and those from the Pacific coast to be still higher. The nitrogen has the reverse relation. The few exceptions which are evident are to be expected.

The analyses show that the seeds from the Southwest tend to be low in gossypol, those from the Southeast somewhat higher, and those from the Pacific coast regions still higher. Even more significant than this is the tendency of the gossypol to follow what may be termed the "rule of the oil." Seeds which are somewhat atypical of the region in which they are grown, as indicated by their oil content, vary correspondingly in their gossypol content. This shows that the gossypol content is closely related to the oil content, and only in a general way to the place of production. The seeds lowest in oil (Lone Star, 1917 or 1918, and Trice, 1918) have the smallest gossypol content, while the seeds highest in oil (Acala from Bakersfield, Calif.) are only 0.2 per cent below the highest in percentage of gossypol (Egyptian seeds).

These results are of interest to plant physiologists. The correlations and variations herein recorded should prove useful in attacking problems dealing with the causes which underlie variation in chemical composition. The possibility of developing a gossypol-free variety of cotton with the retention of the attribute to develop oil, which is at present correlated with the development of gossypol, should be borne in mind. The statement herein made as to the "rule of the oil" should be interpreted to mean the simultaneous correlated appearance of gossypol and oil, and not a cause and effect phenomenon.

SUMMARY

(1) Gossypol was found in the kernels of every sample of cottonseed examined, in Ingenhouzia (Arizona wild cotton), and in a sample of commercial cotton-root bark.

(2) The optical crystallographic properties of gossypol "acetate" are described.

(3) The proportion of gossypol varies in raw cottonseed kernels from about 0.4 to 1.2 per cent, a variation of 300 per cent.

(4) The gossypol content appears to depend upon factors other than varietal factors. If a varietal influence exists, practically it is masked. A variation of 200 per cent was found in samples of one variety from the same plantation, but from crops of different years.

(5) The variation in the gossypol content was fairly regular in that it tended to vary directly with and bore a true relationship to the oil content. This was true for all seeds from any one region, regardless of the regional tendency.

LITERATURE CITED

ALSBERG, C. L., and SCHWARTZE, E. W.
1919. PHARMACOLOGICAL ACTION OF GOSSYPOL. *In* Jour. Pharmacol., v. 13, p. 504. (Abstract.)

———
1919. THE RELATION OF COTTONSEED POISONING TO GOSSYPOL. *In* Jour. Pharmacol., v. 17, p. 344–345. (Abstract.)

CARRUTH, Frank E.
1917. METHODS FOR APPROXIMATING THE RELATIVE TOXICITY OF COTTONSEED PRODUCTS. *In* Jour. Biol. Chem., v. 32, p. 87–90.

———
1918. CONTRIBUTION TO THE CHEMISTRY OF GOSSYPOL, THE TOXIC PRINCIPLE OF COTTONSEED. *In* Jour. Amer. Chem. Soc., v. 40, p. 647–663.

COOK, O. F.
1918. COTTON AS A SEED CROP. *In* Science, v. 48, p. 167–168.

CRAWFORD, Albert C.
1910. A POISONOUS PRINCIPLE IN CERTAIN COTTONSEED MEALS. *In* Jour. Pharmacol., v. 1, p. 519–548. Bibliographical footnotes.

FRAPS, G. S.
1910. COTTONSEED MEAL AS HUMAN FOOD. Tex. Agr. Exp. Sta. Bul. 128, 15 p.

PIPER, C. V., and MORSE, W. J.
1916. THE SOY BEAN, WITH SPECIAL REFERENCE TO ITS UTILIZATION FOR OIL, CAKE, AND OTHER PRODUCTS. U. S. Dept. Agr. Bul. 439, 20 p., 3 fig.

RAST, Loy E.
1918. OIL CONTENT OF COTTON SEED AS INFLUENCED BY VARIETY AND SELECTION. Ga. State Col. Agr. Circ. 70, 4 p.

STANFORD, Ernest E., and VIEHOEVER, Arno.
1918. CHEMISTRY AND HISTOLOGY OF THE GLANDS OF THE COTTON PLANT, WITH NOTES ON THE OCCURRENCE OF SIMILAR GLANDS IN RELATED PLANTS. *In* Jour. Agr. Research, v. 13, p. 419–436, pl. 42–50. Literature cited, p. 434–435.

THOMPSON, H. C., and BAILEY, H. S.
1916. PEANUT OIL. U. S. Dept. Agr. Farmers' Bul. 751, 16 p.

WITHERS, W. A., and CARRUTH, F. E.
1915. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED MEAL. *In* Jour. Agr. Research, v. 5, p. 261–288, pl. 25–26. Literature cited, p. 287–288.

———
1918. GOSSYPOL, THE TOXIC SUBSTANCE IN COTTONSEED. *In* Jour. Agr. Research, v. 12, p. 83–102, 3 fig., pl. 1. Literature cited, p. 100–101.

INHERITANCE OF DWARFING IN MAIZE[1]

By J. H. KEMPTON

Assistant Plant Breeder, Office of Biophysical Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

There are two forms of dwarf plants in maize which seem to be inherited as simple Mendelian characters recessive to normal stature. These two dwarf forms differ in most characters but are alike in that the great reduction in stature is brought about in each case through a reduction in the length and not in the number of internodes. One of these dwarfs is known simply as Dwarf (5)[2]; the other has been designated Brachytic (7).

The variation known as dwarf is one which has been confused until recently with a somewhat similar semidwarf variation now known as anther ear, both being andromonoecious, but that these two variations are wholly unrelated has been demonstrated by the Emersons (5). In view of their rather close resemblance, involving the same complex of characters, and the confusion of the two forms in earlier reports, it is not possible, in the absence of genetic comparisons, to state with certainty which of the two forms has been found by the different observers. One of these andromonoecious types of maize was described by Montgomery (9), who found it in a stock of Stowell's Evergreen sweet corn. Other independent origins have been reported of variations very similar to the one designated dwarf, which seems to be one of the commonest major variations in maize, appearing in wholly unrelated stocks from widely separated localities.

Two of our pedigreed cultures have given rise to andromonoecious plants of dwarfed stature. These cultures were not related, one being a hybrid of the hairy Mexican type, Esperanza (2), with Emerson's liguleless strain (4), and the other a variety of maize originally grown by the Pawnee Indians, the seed stock of which was received from Mr. M. R. Gilmore. In this latter case the variations appeared in the fourth generation of consecutive inbreeding. These variations were dissimilar in size, that from the Pawnee variety being somewhat variable in height but obviously larger than that from the Esperanza-liguleless hybrid.

Comparisons were made between these two dwarf forms and the variations described and named by Emerson, anther ear, and dwarf, seed of which was kindly furnished by Professor Emerson. The strain derived from the Esperanza resembles Emerson's dwarf while the dwarf from Pawnee more closely resembles the anther ear, but both variations with respect to stature occupy an intermediate position between anther ear and dwarf, probably due to the height characteristics of their parental strains. The tallest of the dwarf plants, however, are less than half the height of their normal sibs, and while variation exists among them they never approach in height plants of normal stature (Pl. 1). Without

[1] Accepted for publication May 2, 1923.
[2] Reference is made by number (italic) to "Literature cited," p. 320–321.

Journal of Agricultural Research, Washington, D. C.

Vol. XXV, No. 7
Aug. 18, 1923
Key No. G-325

having tested these variations by interhybridization, it seems certain that at least the smaller of our dwarfs is identical with that known as dwarf by Emerson.

This latter form has been used in the hybrids described in this paper, and to obviate circumlocution is referred to throughout as dwarf. The reader should bear in mind, however, that close somatic resemblance is no certain indication of genetic identity, though the very close similarity of all the teratological characters of these andromonoecious forms raises the question as to whether these variations are not due to some common causes. The andromonoecious dwarfs and semidwarfs seem always to appear as simple Mendelian segregates formed of a complex of characters whose component parts, or at least characters very similar in appearance, are known to occur separately and in different combinations in other nondwarf strains. The most striking characteristic of dwarf is its greatly reduced stature, which often is less than one-fifth that of normal sister plants, although there seems to be no compensating increase in the diameter of the culm. The leaves are reduced in length and increased in width, entirely altering the normal proportions and giving the plants a peculiar tobacco-leaved appearance. Associated with the reduction in stature, there is also a proportional reduction in the size of the tassel, and the number of branches seldom exceeds three. Perfect flowers or staminate spikelets with large anthers are found throughout the ear, which usually terminates in a staminate spike, but, notwithstanding this excess of staminate development, pollen is shed sparingly and the anthers are rarely fully exserted, and even more rarely dehisce. The plants seem exceptionally vigorous and sturdy and the leaves are a very dark green. The reduction in stature is accomplished entirely through a shortening of the internodes—not through a reduction in their number, which remains the same as in normal sister plants. In this respect the plants of dwarf resemble the plants of brachytic, a type of dwarf in which the stature only is reduced (Pl. 2, 3).

The brachytic type also has appeared in unrelated stocks. The chief characteristic of this variation is a shortening of the internodes, which includes also the homologous parts of the tassel, resulting in a reduction of the branching space. This reduction seems unaccompanied by other changes in the tassel, with the possible exception of a slight increase in the number of tassel branches. There are a few other minor changes, such as an increased diameter of the stalk, but nothing of a striking nature comparable to the reduction in stature. The leaves are of the same size and proportion as in normal plants and there is no tendency to produce perfect flowered ears. There is evidence, however, that the brachytic, like the andromonoecious dwarf, is associated with the development of staminate spikes on the ears (*8*).

FIRST GENERATION

In view of their common characteristic of shortened internodes, it might be supposed that crosses between dwarf and brachytic plants would produce nothing but plants of short stature. However, such is not the case, for the first generation of such hybrids consists of normal plants fully as tall as the normal plants from which the immediate brachytic parent is derived, the observed height being 21.0 ± 1.03 dcm. These F_1 plants are also normal with respect to all other teratological characters of their parents. While it is not uncommon to find that

crosses between variations somewhat similar in appearance result in the restoration of the normal form, these two dwarfs are so strikingly alike in the characteristic of reduced internode length that F_1 plants of normal stature were not anticipated.

That the combination of these two dwarf forms should restore completely tall stature furnishes an impressive example of the potential hereditary possibilities resident in abnormal variations and demonstrates the futility of predicting the hereditary behavior of defects which appear similar.

SECOND GENERATION

The distribution to be expected in the second hybrid generation of such a cross, assuming that the two characters are unrelated genetically, is nine normal, three brachytic, three dwarf, and one representing a combination of the dwarf and brachytic forms, the physical characteristics of which can not be predicted from those of the parents. Six rather large progenies were grown, but no group representing the combination of the brachytic and dwarf variation was recognized. The classification of plants is given in Table 1.

TABLE I.—*Showing the number and percentages of the three types of plants obtained in the second generation of the dwarf-brachytic hybrid*

Progeny.	Number of—				Percentage of—	
	Normal.	Brachytic.	Dwarf.	Total.	Brachytic.	Dwarf.
16444 L_1 R21	123	45	36	204	22.1 ±2.00	17.6±1.8
L_2 R21	107	39	17	163	23.9 ±1.90	10.4±1.6
L_3 R21	140	60	28	228	26.3 ±2.00	12.3±1.5
L_4 R21	145	45	6	196	23.0 ±1.90	3.1± .8
L_5 R21	143	40	8	191	20.9 ±2.00	4.2±1.0
L_1 R22	560	172	192	924	18.6 ± .27	20.8± .3
Total	1,218	401	287	1,906	21.04 ± .20	15.1 ± .17

The first five progenies were grown at Arlington, Va., in 1921, and the sixth at the same place in 1922. Unusual care was exercised in 1922, and both soil and weather conditions were much more favorable for the survival of dwarf plants than they were in 1921. In the discussion to follow only plants raised in 1922 are considered.

Subsequent breeding experiments with self-pollinated brachytic plants from these segregating F_2 progenies have shown that the combination of the two variations resembles dwarf plants very closely, being somewhat smaller perhaps, though not strikingly so, and having the accompanying characteristics such as perfect flowered ears, etc. (Pl. 4). In this respect the double recessive form of brachytic-dwarf differs markedly from that found by Emerson in the cross between dwarf and anther ear, where a strikingly small and rather easily identified sterile double recessive was isolated in the second generation.

Sixteen F_3 progenies were grown from self-pollinated seed of brachytic segregates in the F_2 populations of 1921. Of these, just half proved to be heterozygous for dwarf, these dwarf plants representing the double

recessive combination. While two-thirds or 11 of the 16 would be expected to produce dwarf plants if the brachytic and dwarf characters are independent, the departure of 3 from this expectation may be ascribed to chance. Curiously, the percentage of germination was slightly higher in the segregating progenies than in the others, but not significantly so. The percentage of dwarf plants in the 8 progenies was very close to the expected, though 2 were very low. It is interesting to observe that the percentage of germination on the 3 progenies which were below the expected in the percentage of dwarf plants is 13.8 ± 2.1 lower than the progenies which equaled or exceeded the expected percentage of dwarfs. The classification of plants is shown in Table II.

TABLE II.—*Showing F_3 results obtained from growing self-pollinated seed of the brachytic plants which reappeared in the F_2 of dwarf-brachytic; the dwarf plants obtained represent the combination of the two characters, dwarf and brachytic; the counts are made of seedlings raised in greenhouse flats*

PROGENIES SEGREGATING FOR DWARF

Progeny.	Number of—				Percentage of—	
	Seeds planted.	Non-dwarf.	Dwarf seedlings.	Germinated seeds.	Germination.	Dwarf seedlings.
1	108	49	29	78	72.0	37.2±5.3
2	100	73	27	100	100.0	27.0±4.4
3	106	62	19	81	76.5	23.4±4.2
4	100	65	31	96	96.0	32.3±4.8
5	115	52	18	70	60.9	25.7±5.2
6	100	65	9	74	74.0	12.1±3.8
7	100	52	4	56	56.0	7.1±2.8
8	100	60	29	89	89.0	32.6±4.9
Total	829	478	166	644	77.7	25.8±1.7

PROGENIES NOT SEGREGATING FOR DWARF

1	108	96	.....	96	89.0	.....
2	80	48	.....	48	60.0	.....
3	117	110	.....	110	94.0	.....
4	115	93	.....	93	81.0	.....
5	102	93	.....	93	91.0	.....
6	115	90	.....	90	78.0	.....
7	100	10	.....	10	10.0	.....
8	102	99	.....	99	97.0	.....
Total	839	639	.....	639	76.2	.....

The inclusion of the double recessive class in the group of dwarf stature should have been reflected in the ratio of dwarfs to the other groups in the F_2 populations, the expectation then being nine normal, three brachytic, and four dwarf, but it is reasonable to suppose that the relatively high death rate for dwarf plants in field cultures so reduced the percentage of this type that the small increment due to the addition of the double recessive combination did not fully compensate for the loss due to low viability.

When grown under more favorable conditions in greenhouse flats, the percentage of dwarf seedlings, which are recognized easily by their short broad leaves, is found to approximate closely the expected 25. Thus three progenies involving 823 plants gave, respectively, 31.4 ± 2.4, 25.1 ± 1.74, and 21.3 ± 1.43, with a percentage of 24.7 ± 1.02 for the combined totals.

Measurements were made of five characters of 924 plants of the second generation progeny grown at Arlington in 1922. These plants also were classified arbitrarily into the three groups of normal, dwarf, and brachytic stature. In addition, the plants were classed for pericarp color, anthers in the ear, and staminate spikes on the ear. These latter might better have been measured, since they varied greatly in length and in the ratio of the staminate to pistillate portions. The hybrids also involved liguleless leaves and were classed with respect to this character. The biometrical constants of these characters are given in Table III and the percentages of individuals showing them in the three groups and in the total population are given in Table IV.

TABLE III.—*Biometrical constants for the second generation of the dwarf-brachytic hybrid grown at Arlington, Va., 1922*

	Stature.									Total population.		
	Normal.			Dwarf.			Brachytic.					
	Mean.	σ	PE.	Mean.	σ	PE.	Mean.	σ	PE.	Mean.	σ	PE.
Height	23.3	4.00	0.12	4.87	0.99	0.05	8.55	1.97	0.10	16.70	8.86	0.20
Length fifth leaf	77.0	10.30	.30	40.06	8.30	.42	67.66	1.12	.08	72.60	17.90	.42
Width fifth leaf	9.9	1.96	.06	11.20	2.19	.11	8.89	2.04	.13	10.10	2.10	.05
Number tassel branches	18.3	10.50	.30	.69	1.30	.06	20.19	11.40	.65	14.80	12.10	.28
Total number leaves	22.5	1.60	.05	24.70	2.00	.27	23.04	2.02	1.57	22.96	1.99	.06
Leaf index	12.2	3.10	.09	35.17	3.90	.20	12.66	4.51	.29	15.10	6.38	.15

TABLE IV.—*Percentage of plants in the three stature groups having the characters listed in column 1*

Characters.	Stature.						Total population.	
	Normal.		Dwarf.		Brachytic.			
	Per cent.	*PE.*	*Per cent.*	*PE.*	*Per cent.*	*PE.*	*Per cent.*	*PE.*
Brachytic							18.7	0.87
Dwarf							20.8	.91
Intermediates	27.0	1.26	20.3	1.94	27.9	2.3	25.8	.97
Purple flowered ears	.6	.22	100.00	.....	4.51	1.21	22.95	.98
Staminate spikes	26.2	1.28	99.9	.....	18.2	1.45	40.9	1.14
White pericarp	20.9	1.65	22.7	4.2	40.3	4.36	24.1	1.49
Tassels without branches	2.2	.42	67.8	2.3	2.9	.58	16.2	.84

INHERITANCE OF SIZE CHARACTERS

As was to be expected, the height of the plants as measured in decimeters showed a trimodal distribution, the dwarf and brachytic plants forming a group at the low end of the scale. The frequency distribution for this character is shown in figure 1 and their relative heights are shown in Plate 5.

In the cross between dwarf and anther ear, reported by the Emersons, where normal stature also was restored in the F_1, the distribution with respect to height shows the anther ear and normal segregates to be more

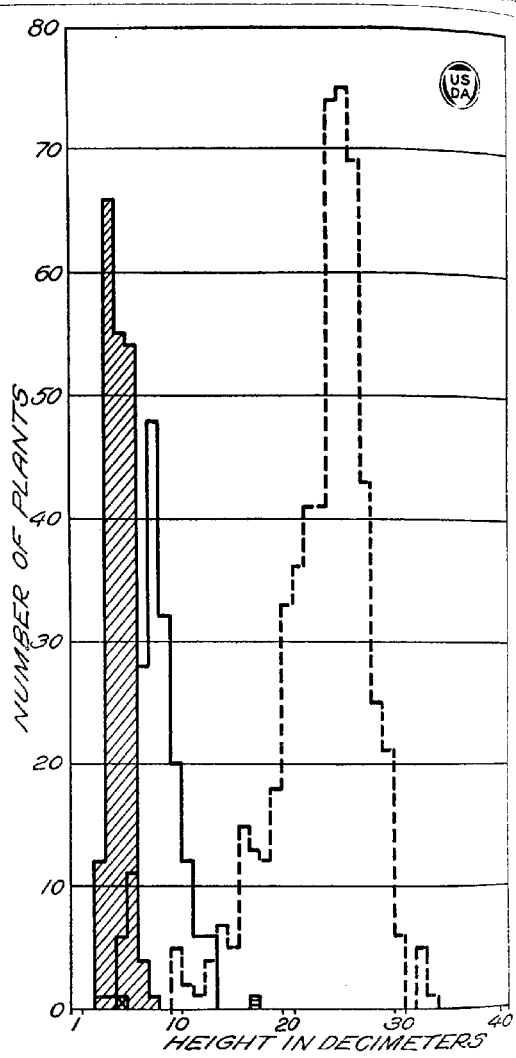


FIG. 1.—Frequency distributions for plant height. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants. The square marked X represents a plant classed as normal; the square with horizontal lines represents a plant classed as brachytic.

nearly of the same height, while the dwarf segregates are much smaller with no overlapping (5). This is different from the brachytic-dwarf hybrid, where the brachytic and dwarf segregates form one group and the normals the other. An examination of the height data shows that brachytic occupies with respect to stature a position intermediate between anther ear and dwarf, approaching the dwarf stature rather more closely

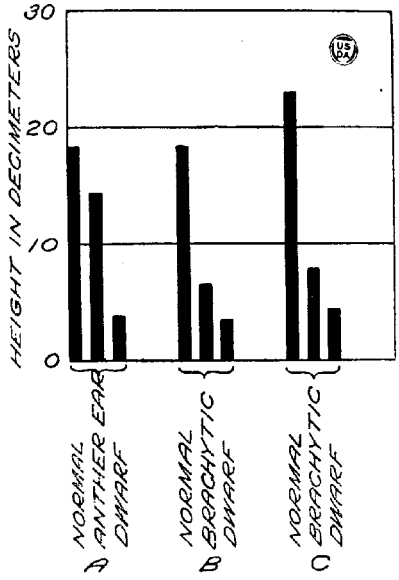


FIG. 2.—Comparison of the plant heights of normal, anther ear, and dwarf as grown by the Emersons, with normal, brachytic, and dwarf from the dwarf-brachytic hybrid. A, Average height of Emersons' normal, anther ear, and dwarf plants. B, Average height of normal, brachytic, and dwarf plant from the dwarf-brachytic hybrid reduced in proportion to the difference between the normal segregates of the two hybrids. C, Average height of normal, brachytic, and dwarf plants from the dwarf-brachytic hybrid.

than the anther ear. This relationship is shown diagrammatically in figure 2.

The entire population of the brachytic-dwarf hybrid was taller than that of Emerson's anther ear dwarf, but in reducing each stature group of the brachtyic-dwarf hybrid in proportion to the difference between the normal segregates of the two hybrids, the groups of dwarf stature are found to be very similar, the mean height being 3 and 3.87, respectively, while the mean height of the brachytic group becomes 6.8 as compared with 14.8 for the anther ear stature.[3]

[3] Three F_2 populations are shown in the Emersons' paper. Two of these populations are very similar in the mean height of the three groups, while the other (the first) is somewhat smaller. In making the height comparisons, the group having the tallest plants of normal stature was chosen; this population also had the largest number of individuals but the relationships of the three height groups in anther-ear dwarf to those of the brachytic-dwarf would remain very much the same irrespective of which of the three anther-ear dwarf groups was selected.

The length of the fifth leaf from the top also showed a good bimodal distribution with the brachytic plants, forming a somewhat intermediate grouping, as shown in figure 3.

The width of the fifth leaf was strictly unimodal, with the dwarf plants grouped at the upper end of the scale. There was little difference between the normal and brachytic plants with respect to this character, as is shown in figure 4.

From the length and width of the fifth leaf it was possible to formulate an expression for leaf shape. This figure has been designated leaf index and was obtained by dividing the width by the length.

The distribution of plants with respect to leaf shape was bimodal, with the dwarf class well grouped at the upper end of the scale and the brachytic and normal plants occupying the lower end as shown in figure 5.

With respect to the number of tassel branches, 68 per cent of the dwarf plants had no branches and the highest number of tassel branches found

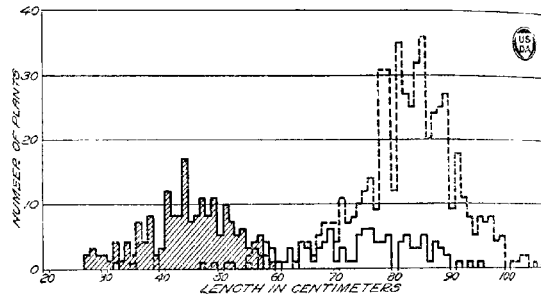


FIG. 3.—Frequency distribution for length of fifth leaf. Shaded portion, dwarf plants; solid line, brachytic plants; broken lines, normal plants.

in this group was 6, while the brachytic plants ranged from 0 to 37 branches and the normals from 0 to 44, the latter forming a fairly regular distribution, as is shown in figure 6.

The brachytic and dwarf plants differed little in total number of leaves, though both had an appreciably higher number than the normal plants. The distribution, however, was unimodal, as is shown in figure 7.

The relative differences between the segregates with respect to all measured characters is shown in figure 8.

From the character of these distributions it would seem that in the case of the dwarf variation a relatively few hereditary elements will account for the differences between this and the normal form. The behavior of the leaf lengths and shapes is very different from that encountered where short, broad leaves of nondwarf stock are crossed with relatively long, slender leaves of some other strain. In such cases the frequency distributions are unimodal, the indication being that several hereditary factors are concerned in the differences between the parents.

CORRELATIONS OF MEASURED CHARACTERS

The character of the distributions in most cases precludes the use of the correlation coefficient, since the measured character so often is bimodal. Recourse may be had to fourfold groupings, dividing the population arbitrarily into two groups of the measured character, but when such

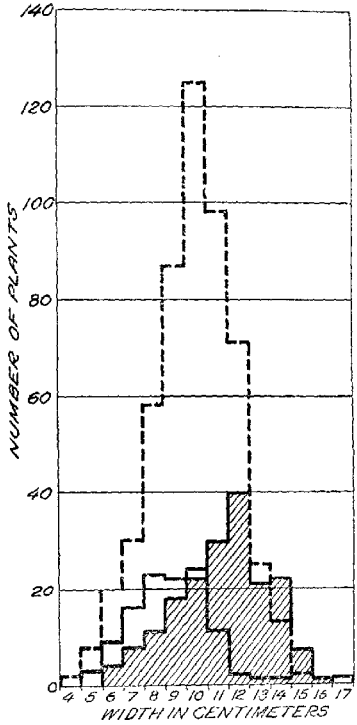


FIG. 4.—Frequency distribution for width of leaf. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

divisions are made with due regard to the character of the distributions one class is often zero or very small. Under such conditions a correlation coefficient is practically without meaning, and such coefficients have not been calculated. In those cases where the data justified the use of the biserial correlation, the coefficients have been calculated and are given in Table V. For the most part, however, the frequency polygons will give a clear conception of the nature of the inheritance.

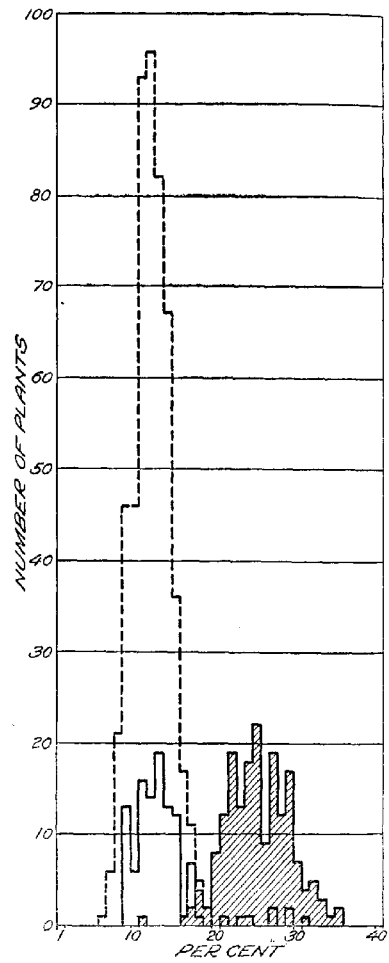


FIG. 5.—Frequency distribution for leaf index. Shaded portion, dwarf plants; solid lines, heterozygous plants; broken lines, normal plants.

TABLE V.—*Coefficients of biserial correlations in the second generation of the dwarf-brachytic hybrid*

Measured characters.	Stature groups.		
	Normal versus brachytic stature.	Normal versus dwarf stature.[b]	Dwarf versus brachytic stature.
Length leaf	[a] −0.40±.033		
Width leaf	[a] −.20±.037	0.37±.029	0.431±.037
Number tassel branches	.12±.036		
Total number leaves	.75±.045	.09±.063	[c] −.099±.054
Leaf index	.22±.037		
Height			[c] −.882±.035

[a] Minus sign indicates negative correlation with brachytic stature.
[b] Correlations are positive with dwarf stature.
[c] Minus sign indicates negative correlation with dwarf stature.

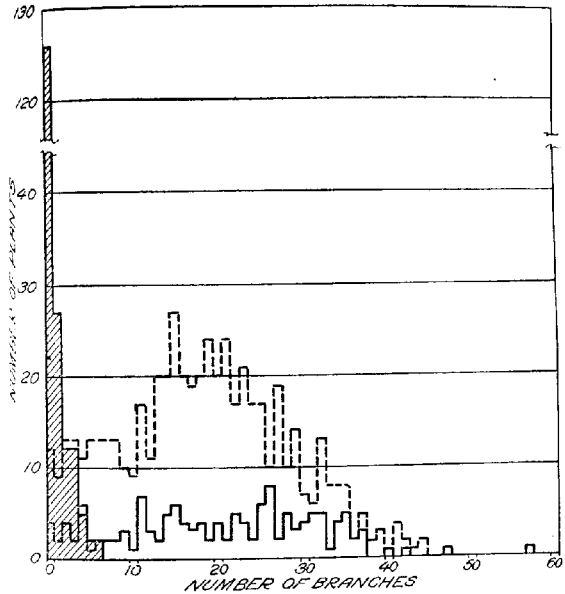


FIG. 6.—Frequency distribution for number of tassel branches. Shaded portion, dwarf plants; solid lines, brachytic plants; broken lines, normal plants.

While the heterogeneous nature of the F_2 plants precludes the possibility of analyzing the correlations of the measured characters in the population as a whole, some insight may be gained on the interrelations of these characters if the three groups of plants, normal, brachytic, and dwarf, are analyzed separately. These correlations are given in Table VI.

In each of the three groups of stature, tall plants are found associated with long leaves, a relation to be expected from the standpoint both of physiology and genetics. It is of interest, however, that the relationship is no closer, especially in the group of normal stature.

With leaf width, height is found to be negatively correlated in the brachytic group, while the coefficients of correlation in the other two groups are essentially the same as for leaf length. The negative correla-

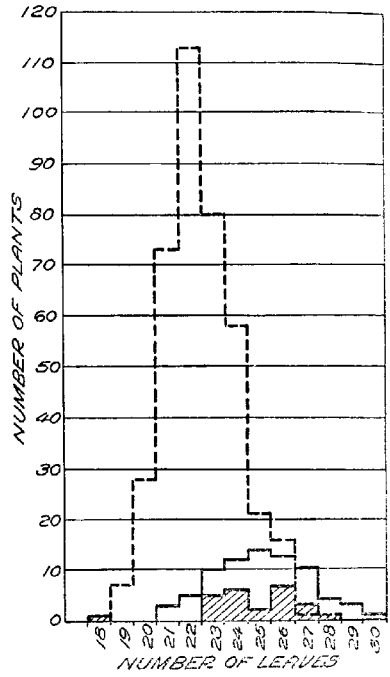


FIG. 7.—Frequency distribution for total number of leaves. Shaded portion, dwarf plants; solid lin brachytic plants; broken lines, normal plants.

tion in the brachytic group indicates that some sort of segregation of th dwarf stature and wide leaves is taking place in this group, since a pos tive correlation would be expected if the association were due to phys ological causes. The correlation with leaf index indicates that th leaves were broad, not only absolutely but relatively, this fact being i the nature of a substantiation of the hypothesis that some of the plant classed as brachytic were potentially dwarfs with respect to these cha acters.

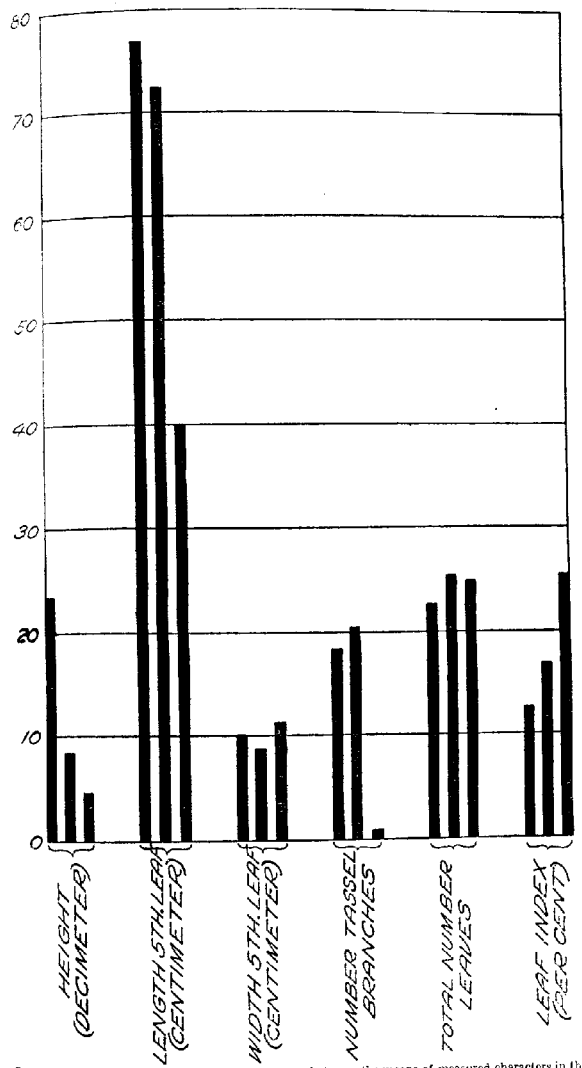


FIG. 8.—Diagrammatic representation of the difference between the means of measured characters in the second generation of the dwarf-brachytic hybrid. Normals at left, brachytic in center, dwarf at right.

TABLE VI.—*Correlation coefficients of the measured characters in the three groups of F plants of the dwarf-brachytic hybrid, normal, brachytic, and dwarf*

	Height.	Length fifth leaf.	Width fifth leaf.	Number tassel branches.	Total number leaves.	Leaf index.	Stature.
Height.		0.215 .434 .441	0.217 −.281 .399	−0.045 −.071 .164	0.161	0.109 −.476 −.045	Normal.[1] Brachytic.[2] Dwarf.[3]
Length, fifth leaf.	.215 .434 .441		.255 −.161 .620	.241 .462 .205	−.100	−.393 −.672 −.261	Normal. Brachytic. Dwarf.
Width fifth leaf.	.217 −.281 .399	.255 −.161 .620		.422 .189 .148	.118	.618 .782 .496	Normal. Brachytic. Dwarf.
Number tassel branches.	−.045 −.071 .164	.241 .462 .205	.422 .189 .148		.032	.197 −.192 −.148	Normal. Brachytic. Dwarf.
Total number leaves.	.161	−.100	.118	.032		.142	Normal.
Leaf index.	.109 −.476 −.045	−.393 −.672 −.261	.618 .782 .496	.197 −.192 −.148	.142		Normal. Brachytic. Dwarf.

[1] In the group of normal stature coefficients above 0.103 are greater than three times their error.
[2] In the group of brachytic stature coefficients above 0.190 are greater than three times their error.
[3] In the group of dwarf stature coefficients above 0.147 are greater than three times their error.

With no genetic interference, wide leaves are found to be correlated with tall plants. In the present hybrid the parental combinations were the reverse of this association and the short plants had the wide leaves.

When the biserial correlations of type of plant with leaf width are examined it is found that as between normal and dwarf stature, short stature is correlated with wide leaves, 0.37 ± 0.029, and as between brachytic and dwarf short stature is associated with wide leaves, 0.43 ± 0.037. When the two stature groups, normal and brachytic, are compared it is found that tall stature is correlated with wide leaves, 0.2 ± 0.037.

As stated above, the product moment correlation between stature and leaf width in the brachytic group is -0.281 ± 0.045 between tall stature and wide leaves. Obviously, such an association can be only genetic, since a normal physiological behavior would lead to a positive correlation between tall plants and wide leaves. There can be little doubt that a genetic correlation of this sort must be reduced in degree by an amount proportional to the positive correlation expected for physiological reasons. In this case the normal interaction of physiological factors in the brachytic group would be expected to be at least as high as that found in the normal group, which is 0.217 ± 0.033, so that the effect of the genetic factors must be the difference between the correlation observed in the normal group and that found in the brachytic group, or 0.498, which is a rather high degree of relationship.

Such a result indicates that in the brachytic group there are segregating height factors resembling dwarf in that they are associated also

with factors for broad leaves. This would be brought about if dwarf plants were included among the brachytic plants in the classification into groups. The frequency polygon for stature in the brachytic group shows clearly that there is no bimodality, and an examination of the short, broad-leaved plants in this group fails to show any of the other dwarf characters, such as few tassel branches or perfect flowered ears, eliminating the possibility that the plants were classified improperly.

A further substantiation of the dwarflike characteristics of certain brachytic plants is found in the negative correlation between length and width of leaf, a correlation which is positive in both the normal and dwarf groups. Almost as unexpected is the rather high positive correlation of length with width of leaf in the dwarf group, the group which as a whole has short wide leaves. The restoration of the normal physiological correlation indicates that there is no segregation of varying degrees of dwarfness as such in this group, but rather that such variation as exists is due to the effects of environment or possibly to unrelated modifying factors.

The correlation of width of leaf with number of tassel branches in both the dwarf and brachytic groups are much smaller than the correlation in the normal group, though all three are positive. The coefficients in the brachytic and dwarf groups do not depart significantly from zero, while the coefficient in the normal group clearly is significant. The fact that the normal physiological relationship of wide leaves and many tassel branches has been largely reduced in the brachytic and dwarf groups indicates that a genetic cause is involved, an indication which derives support, of course, from the fact that the dwarf parent represents a condition where not only relatively wide but actually wide leaves are associated with few tassel branches.

The number of tassel branches shows no other unusual relationhips. The correlations with total number of leaves were not calculated for other than the normal group, since on both brachytic and dwarf plants a large number of leaf tags were lost.

Thus the population of these groups was reduced, and since the loss of tags was in inverse proportion to the height of the plants a selective action was involved which might result in spurious relationships so that correlation coefficients would be of little value if calculated.

INHERITANCE OF TERATOLOGICAL VARIATIONS

STAMINATE EAR SPIKES

The percentage of plants with staminate ear spikes in the whole F_2 population, including normal, brachytic, and dwarf plants, is 40.9 ± 1.14. The three groups of stature—normal, brachytic, and dwarf, had, respectively, 26.2 ± 1.28, 18.2 ± 2.27, and 99.4 ± 0.4 per cent of the plants with staminate spikes.

The difference in percentage of this character between the normal and brachytic groups is 8.0 ± 2.61, or 3.06 times the error. Yule's coefficient of association between normal stature and the development of staminate spikes in the normal-brachytic population is only 0.229 ± 0.074, while the departure from a 50 per cent crossover ratio as measured by χ^2 is 3.71. It may be concluded, therefore, that the brachytic and normal groups are alike with respect to the development of staminate ear spikes, both approximating 25 per cent. The hypothesis has been advanced

previously that the development of staminate ear spikes is dependent upon the interaction of two factors, the character appearing when either or both factors are homozygous recessive (*8*). In addition, one of these factors is associated or linked with brachytic, while the other is independent of stature.

The percentages of this character in the three stature groups of the present hybrid necessitates the assumption of a third element for the production of staminate spikes. This third element is linked closely with dwarf stature.

Such characters are generally considered as multiple factor characters comparable with those which come into expression only when all the factors are homozygous recessive. There is little to justify such a classification except perhaps an inability to distinguish somatic differences, an inability which admittedly is personal.

With cases such as the aleurone color of the seeds, where all white seeds have very much the same shade, there is little hope of distinguishing a difference between the several factors and little is to be gained by considering each of the several forms of genetic whites as separate monohybrid characters. With other characters where distinctions are not made in the beginning they are often recognized later, and the variations are then classified as independent monohybrid characters.

It would seem best to consider those characters which come into expression when single or when each of several factors is homozygous recessive, as distinct monohybrid characters, even though they can not be distinguished readily; whereas those characters which appear only when more than one factor is homozygous recessive are true multiple factor characters. With the former each variation in the germ plasm results in a visible somatic change, while in the latter a somatic change results only from the cumulation of several variations in the germ plasm.

It is obvious that a cross involving two independent monohybrid characters which are indistinguishable in appearance would result in the familiar 9:7 dihybrid ratio in the second generation, and the present hybrid may illustrate such a case.

It is clear that the dwarf-brachytic hybrid is homozygous dominant for the factor for staminate ear spike which is linked with brachytic and heterozygous for a factor independent of stature. If this were all, then monohybrid ratios would be expected for the entire population as well as for each stature group, but if a second staminate ear spike character were involved, the gene for the latter being identical or closely linked with dwarf stature, the observed percentages would be approximated closely.

On this hypothesis 25 per cent of the plants in both the normal and brachytic groups would be expected to have staminate ear spikes while all the plants of dwarf stature would have this character, and the percentage for the total population would be 43.75, provided the three stature groups were present in the expected ratio of 9:3:4.

If this hypothesis be true, there are then four staminate ear spike characters, all similar in appearance but distinct genetically. One of these is linked closely with dwarf stature, one is associated with the anther ear semidwarf of Emerson, one is linked with brachytic culms, and the other seems independent of stature.

Variations such as these, strikingly similar in appearance, which prove to be distinct genetically when crossed, are being found with increasing frequency in maize. When such variations affect a similar and peculiar

combination of characters, as is the case with anther ear and dwarf or dwarf and brachytic, the question of a common cause can hardly be avoided. From the mode of inheritance of these characters there can be no doubt that independent genetic changes have taken place on separate chromosomes. That such strikingly similar somatic resemblances can arise as the result of the alteration of unrelated and wholly separate hereditary elements appears incredible. It seems more probable that in maize some or all of the 10 chromosomes are practically identical, each with hereditary elements for all the characters of the complete plant as suggested by Emerson (3). Such a hypothesis permits the prediction that all characters in maize eventually will be found to involve at least 10 independent factors and that these factors in a general way will have similar linkage relations.

Thus the shortened internodes of dwarf and brachytic are genetically distinct, and both are associated with the development of staminate ear spikes. These spikes, like the shortened internodes, though bearing a close resemblance to each other, are wholly distinct from a genetic standpoint. Even more striking, of course, is the character complex of anther ear and dwarf. It seems not unreasonable that a change of whatever form in a similar group of genes in separate chromosomes would result in a somewhat similar somatic behavior. Such a hypothesis is in accord with the fact that most linked groups involve diverse organs and that the factors or hereditary elements for multiple factor characters are not found in one chromosome but are distributed through many.

If other organisms possess identical chromosomes, then those organisms with few chromosomes should have relatively simple characters, speaking in a genetic sense, while those with many chromosomes should have complex characters composed of many factors. If mutations occur at the same rate in organisms with few chromosomes, as in those with many, then those organisms with few chromosomes should have many multiple allelomorphs while those with many chromosomes should have multiple factor characters.

PERFECT FLOWERED EARS

Perfect flowered ears always have been found associated with the dwarf stature in its numerous appearances. The character is of more than usual interest since it represents a reversion toward a more primitive form of maize, comparable in some respects to the ramose and tunicate forms. Perfect flowered ears are not limited to dwarf plants, and many maize breeders have encountered them on plants otherwise quite normal. In the writer's experience the character is ephemeral, repeated inbreeding failing to stabilize its appearance in stocks of normal stature; and since, strictly speaking, it is the development of vestigial floral organs, it may well be that environment, especially photoperiodism, which is recognized as having a profound influence on the development of sex organs, is an important factor in its expression.

In the present hybrid, 22.95 + 0.98 per cent of the plants were found to have perfect flowered ears. This seems fairly close to the expected 25 per cent and it may be accepted as a simple Mendelian character. When the three classes of plants—normal, brachytic, and dwarf—are examined with respect to this character, it is found that of the normal plants only 0.6 ± 0.22 per cent have perfect flowered ears, while of the

brachytics 4.5 ± 1.2 and of the dwarfs all have perfect flowered ears. The distributions are:

	Pistillate ears.	Perfect flowered ears.	Total.	Per cent.
Normal stature	532	3	535	0. 56 ± 0. 22
Brachytic stature	126	6	132	4. 54 ± 1. 2
Dwarf stature	0	187	187	100. 00 ±
Total	658	196	854	23. 0 ± . 9

Combining the brachytic and normal stature groups, which differ only slightly in excess of three times the error in the percentage of plants with perfect flowered ears, the resulting fourfold distribution is:

	Pistillate ears.	Perfect flowered ears.	Total.
Nondwarf	658	9	667
Dwarf	0	187	187
Total	658	196	854

Looked at as closely linked but distinct characters, Haldane's P = 0.9888 with the percentage of crossover 1.12 (*6*). The expected distribution on the assumption of a 1.12 per cent crossover is:

Expected	636. 3	4. 2	4. 2	209. 3
Observed	658. 0	0	9	187. 0
	22. 0	4. 2	4. 8	22. 0

$\chi^2 = 12.75$; this is a rather poor fit, but in this case the departure from the Mendelian percentages are being measured also, and there is direct evidence that the population has been reduced through the low viability of the dwarf plants. With this knowledge, it seems quite fair to calculate the expected on the basis of the actual percentage of dwarf plants found, since the end result will afford an opportunity to determine whether the absence of crossover plants in the dwarf group is of any significance.

The distributions, taking into account the low percentage of dwarf plants, become—

Calculated	661. 1	6	3. 7	183. 3	
Observed	658. 0	9	0	187. 0	
	3. 1	3	3. 7	3. 7	($\chi^2 = 5.287$

The fit is rather better, and it seems safe to conclude that the genes for dwarf stature and perfect flowered ears are located in the same chromosome with approximately 1 per cent of crossing over.

The failure to find a crossover class involving the short stature of dwarf suggests that such combinations fail to survive, though such an hypothesis seems wholly without a logical basis, since all crossovers in this group would be in the direction of normal plants and, therefore, theoretically have a higher survival value than the noncrossover, unless the assumption is made that some or one of the characters associated with dwarf stature is closely linked with dominant factors for growth, and hence the crossovers with stature would have still a lower survival value than the dwarf complex. In some aspects this hypothesis seems worthy of consideration.

Lethal or semilethal characters that recur constantly must be due either to frequent mutation or, as seems more probable, must be associated with some hereditary element that raises the survival value of the heterozygous stock. While mutations are known to occur in certain stocks with frequency, if such mutations have a negative survival value only, it is difficult to see how they and their mutating stocks persist, since their lethal nature insures their rapid elimination. That stocks of maize heterozygous for lethal characters are common is well known. Perhaps the most widely recognized of these is the albino, or white seedling, which, of course, never produces seed. There are others not so well understood but equally lethal, while the number of semilethals seems legion. Perhaps the most common of these, from the standpoint of repeated occurrence, is the andromonoecious dwarf. If, as has been suggested, maize possesses dominant factors favorable for growth, the problem of the recurrence of lethal factors is simplified by predicating that the deleterious variations which reappear frequently are those closely linked with one or more of these factors for growth, though the mutations need not have arisen in the chromosomes with the favorable growth factors since occasional crossovers would permit their survival. On this hypothesis it seems clear that lethal or semilethal variations must be those closely linked with the factors favorable for growth and, therefore, though destined for death in the homozygous condition, have a survival value as heterozygotes higher than that of normal plants. If this be true, then in a stock heterozygous for lethal or semilethal factors, the homozygous normal plants should be inferior in reproductive value to the heterozygous ones.

Breeders long have recognized that certain variations have a sturdy, vigorous appearance which belies their inherent defects. Combinations of such variations usually make very favorable first generation hybrids, and it should be possible to reconstruct a high yielding strain by combining the deleterious recessive variations. If this be true generally, the practice of inbreeding and discarding those progenies which show deleterious Mendelian variations is resulting in the elimination from the stock of the most desirable hereditary elements.

The survival value of dwarf plants under field conditions is so low that a higher death rate for the crossover classes in this group could pass unnoticed, and of course there is no possibility of recognizing them in the seedling stage.

In an unrelated hybrid between Esperanza and liguleless, the combination of pistillate ear and dwarf stature was obtained. The fourfold distribution in this hybrid is:

Normal stature, pistillate ears.	Normal stature, perfect flowered ears.	Dwarf stature, pistillate ears.	Dwarf stature, perfect flowered ears.
210	10	4	48

The percentage of perfect flowered ears is 21.3 ± 1.67 and the percentage of dwarf plants is 19.1 ± 1.6, while $Q = 0.992 \pm 0.004$, indicating about 5 per cent of crossing over.

LIGULELESS LEAVES

The percentage of plants with liguleless leaves in the entire population was 25.8 ± 0.97, while the percentage in the normal group was 27.0 ± 1.27 in the brachytic 27.9 ± 2.3, and in the dwarf 20.3 ± 1.94. Since the percentage for the entire population closely approximates the expected, the low percentage of liguleless plants in the dwarf group can not be charged to a low survival value of the dwarf-liguleless combination. There is little evidence, however, of a linkage, since $Q = 0.2 \pm 0.06$ between dwarf stature and normal leaves, the parental class being dwarf stature and liguleless leaves. The percentage of liguleless plants in the combined normal and brachytic group is 27.2 ± 1.1, from which 20.3 ± 1.94 differ by 6.9 ± 2.23, or but slightly in excess of three times the error.

While there seems to be no association between stature and liguleless leaves, there is a pronounced correlation between liguleless leaves and few tassel branches. This correlation is found in all three of the stature groups, being greatest in the brachytic and least in the dwarf. Such a correlation is in the nature of a coherence and may indicate a linkage between these characters, one of which is certainly very closely associated with dwarf stature. The frequency distribution for number of tassel branches with respect to normal and liguleless plants of tall stature is shown in Figure 9.

TABLE VII.—*Biserial correlations with liguleless leaves in the second generation of dwarf brachytic*

Characters correlated with liguleless.	Population of—		
	Normal stature.	Brachytic stature.	Dwarf stature.
Height of plant	[a] -0.097 ± 0.023	0.031 ± 0.040	0.193 ± 0.0
Length fifth leaf	$-.015 \pm .023$	$-.404 \pm .048$	$-.349 \pm .0$
Width fifth leaf	$-.339 \pm .023$	$-.306 \pm .047$	$-.302 \pm .0$
Number of tassel branches	$-.500 \pm .023$	$-.629 \pm .045$	$-.274 \pm .0$
Total number of leaves	$-.009 \pm .027$	$.116 \pm .061$	$.269 \pm .1$
Leaf index	$-.191 \pm .023$	$-.093 \pm .048$	$-.227 \pm .0$

[a] Minus sign indicates a negative correlation with liguleless leaves.

Negative correlations are indicated between leaf width and liguleless leaves in all three groups, and in the brachytic and dwarf groups a correlation with length of leaf is found. The correlation with leaf width is in

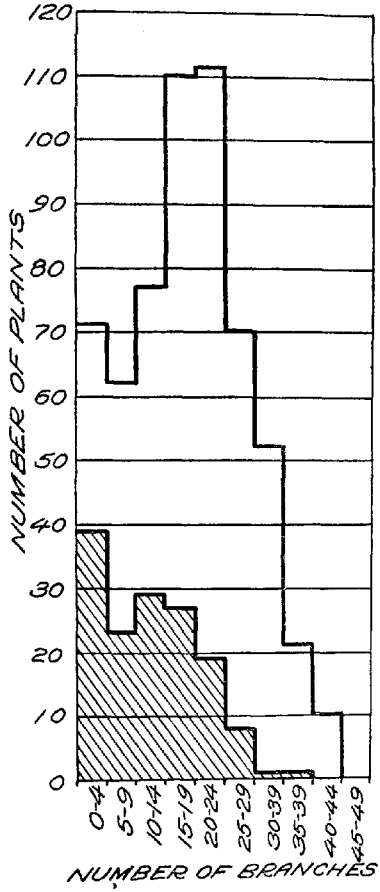


FIG. 9.—Frequency distributions with respect to number of tassel branches on liguleless and nonliguleless plants. All plants of normal stature. Shaded portion, liguleless plants; mean normal, 20.6±0.33; mean liguleless, 11.8±0.45; difference, 8.8±0.56; D/E=15.7; biserial $r_{bis}=-0.50$.

the nature of a disherence, since the parental combination was liguleless and wide-leafed. Disherences of this nature where deleterious variations are combined with characters of diminished size indicate some

physiological cause. Thus, crosses between short plants with broad leaves and tall plants with narrow leaves usually show in the F_2 a correlation between tall plants and wide leaves—a correlation easily understood. Some such explanation may apply in other cases of difference where the physiological relationships are not indicated so clearly, but are working at cross purposes with genetic relationships. The correlation coefficients are given in Table VII and the biometrical constants in Table VIII.

TABLE VIII.—*Biometrical constants for plants with liguleless and normal leaves in the three stature groups, normal, brachytic, and dwarf*

NORMAL STATURE

	Normal leaves.			Liguleless leaves.			Difference.	D.E.
	Mean.	σ	PE.	Mean.	σ	PE.		
Height	23. 11	3. 81	0. 13	23. 77	4. 54	0. 25	+0. 66±0. 29	2. 3
Length fifth leaf	77. 10	9. 85	. 34	76. 80	10. 83	. 63	−. 30± . 72	. 4
Width fifth leaf	10. 19	1. 95	. 13	9. 07	1. 76	. 10	−1. 12± . 16	7. 0
Number tassel branches	20. 62	10. 29	. 33	11. 80	8. 14	. 45	−8. 82± . 56	15. 7
Total number leaves	22. 52	1. 57	. 10	22. 28	1. 54	. 11	−. 24± . 15	1. 6
Leaf index	12. 47	2. 11	. 15	11. 48	4. 91	. 29	−. 99± . 33	3. 0

BRACHYTIC STATURE

Height	8. 21	1. 92	0. 11	8. 31	2. 66	0. 25	+0. 10±0. 27	0. 4
Length fifth leaf	72. 23	10. 04	. 67	61. 30	14. 30	1. 97	−10. 93±2. 08	5. 3
Width fifth leaf	9. 42	2. 14	. 14	8. 24	2. 12	. 29	−1. 18± . 32	3. 68
Number tassel branches	23. 08	10. 10	. 66	10. 94	9. 95	1. 35	−12. 14±1. 50	8. 10
Total number leaves	24. 95	2. 04	. 18	25. 35	1. 89	. 31	+. 40± . 36	1. 11
Leaf index	14. 26	5. 92	. 40	13. 29	5. 96	. 82	−. 97± . 91	1. 06

DWARF STATURE

Height	4. 80	0. 98	0. 06	5. 14	1. 03	0. 12	+0. 34±0. 13	2. 62
Length fifth leaf	45. 93	6. 17	. 36	38. 40	7. 68	. 91	−6. 53± . 98	6. 66
Width fifth leaf	11. 39	2. 22	. 13	10. 19	1. 83	. 22	−1. 20± . 26	4. 62
Number tassel branches	. 8	1. 35	. 08	. 19	. 59	. 07	−. 61± . 11	5. 54
Total number leaves	24. 52	2. 18	. 34	25. 50	1. 66	. 56	+. 98± . 65	1. 51
Leaf index	25. 47	3. 73	. 22	23. 88	. 43	. 43	−1. 59± . 48	3. 31

There seems to be no association between perfect flowered ears and liguleless leaves. The fourfold distribution for these characters is:

Normal leaves.		Liguleless leaves.		Total.
Pistillate ears.	Perfect flowered ears.	Pistillate ears.	Perfect flowered ears.	
451	158	160	40	809

The percentage of liguleless plants is 24.7 ± 1.02, of perfect flowered ears 20.8 ± 0.97, and Q (Yule's coefficient of association) $= 0.167$. The expected distribution on the assumption of a 9:3:3:1 grouping would be 455.1 : 151.7 : 151.7 : 50.5, from which the observed departs by an amount which could be expected as the result of chance about 4 times in 10, ($\chi^2 = 2.94$).

The fact that all but two of the dwarf plants had staminate ear spikes, and the possibility that the absence of such spikes in these two cases may be due to the activities of the ear worm, necessitates the conclusion that this character and dwarf stature are very closely linked. It necessarily follows that the linkage relations of the staminate ear spike character in question will be identical with those of dwarf stature. But by confining the analysis of staminate ear spikes to plants of nondwarf stature, it becomes possible to measure the linkage relations of a single factor for the other heterozygous staminate ear-spike character involved in this cross.

This factor is the one not linked with brachytic stature. Confining the analysis to those plants of normal stature only, the fourfold grouping with liguleless leaves becomes:

Normal leaves.		Liguleless leaves.		Total.
Without ♂spikes.	With ♂spikes.	Without ♂spikes.	With ♂spikes.	
395	140	109	33	677

The percentage of plants with staminate ear spikes is 25.6 ± 1.13 and for liguleless leaves 21.0 ± 1.05, while $Q = 0.079 \pm 0.07$, or practically 50 per cent, of crossing over.

It may be concluded, therefore, that the gene for liguleless leaves is independent of this factor for staminate ear spikes, as well as of the corresponding factor which is linked with dwarf.

INHERITANCE OF PERICARP COLOR

The inheritance of pericarp color in this hybrid presents no new or unexpected features. The association of brachytic stature with colorless pericarp confirms previous results where these characters were found to lie from 31 to 43 units apart. The coefficient of association is found to be 0.389 ± 0.076, which for the 13 to 3 grouping is the equivalent of 38 per cent ± 2.3 crossovers.

SUMMARY

(1) There are two forms of dwarf maize in which the reduction in height is due to shortened internodes and not to a reduction in the number of internodes. One of these is known as dwarf, the other as brachytic. The variation known as dwarf also departs from the normal in characters other than stature. The most striking of these other changes are the shortened and widened leaves, the reduced number of tassel branches, and the perfect flowered ears.

(2) When the dwarf and brachytic variations are crossed, the plants of the first generation are fully as tall as normal plants from which the

brachytic variation arose. These F_1 plants are normal also with respect to the other teratological characters.

(3) In the prejugate generation three types of plants with respect to stature are found—normal, brachytic, and dwarf. These three stature groups occur in the ratio of 9:3:4, when due allowance is made for the low survival value of dwarf plants, indicating that the double recessive, a combination of dwarf and brachytic, closely resembles the dwarf parent. This indication is confirmed when the self-pollinated seed of segregated brachytic plants is grown.

(4) As was to be expected, some of the F_2 brachytic plants proved to be heterozygous for dwarf, their progenies comprising two types of plants—brachytics and dwarfs. These dwarfs represent the double recessive, being a combination of brachytic and dwarf, but are only slightly smaller than the dwarf parent of the original hybrid. They are found to have all the other characteristics of dwarf plants.

(5) From their behavior it may be concluded that dwarf and brachytic are two independent variations, both expressed in reduced stature, the genes for which are located in separate chromosomes.

(6) The analysis of the plants of the second generation with respect to all the characters which differentiate dwarf plants from the other groups shows clearly that the complex of characters associated in the dwarf variation is not inherited invariably as a unit. Thus, many of the plants of normal stature are found to have ears terminating in staminate spikes, or tassels with few or no branches, or short, broad leaves, and even a few were found with perfect flowered ears. Such behavior would indicate that the combination of characters comprising the dwarf variation formed a linkage group with very low crossover values between some of the members. On the other hand, the plants of extremely short stature, easily distinguishable from the brachytic and normal plants as dwarfs, always had other dwarf characters, such as short, broad leaves, perfect flowered ears, and few tassel branches. These dwarf segregates, like their parent, also shed little pollen, though some of the F_2 segregates seem to have improved slightly in this respect.

(7) From a consideration of the general features of such variations as anther ear, dwarf, and brachytic it is suggested that in maize, at least several and possibly all the chromosomes are identical, each having a complete assortment of genes for all the characters arranged in a similar order. Such a condition would arise through a reduplication of the chromosome number in much the same manner as that observed by Blakeslee in Datura (*1*).

(8) The recurrence of degenerative variations is discussed and the hypothesis advanced that the survival of such stocks is due to the linkage relations of deleterious characters with factors favorable for growth.

LITERATURE CITED

(1) BLAKESLEE, Albert F.
1921. TYPES OF MUTATIONS AND THEIR POSSIBLE SIGNIFICANCE IN EVOLUTION. *In* Amer. Nat., v. 55, p. 254–267. Bibliography, p. 266–267.

(2) COLLINS, G. N.
1918. TROPICAL VARIETIES OF MAIZE. *In* Jour. Heredity, v. 9, p. 147–154, fig. 1–3.

(3) EMERSON, R. A.
1911. GENETIC CORRELATION AND SPURIOUS ALLELOMORPHISM IN MAIZE. In Nebr. Agr. Exp. Sta. 24th Ann. Rpt., p. 59–90, 9 fig.

EMERSON, R. A.
1912. THE INHERITANCE OF CERTAIN "ABNORMALITIES" IN MAIZE. *In* Ann. Rpt. Amer. Breeders' Assoc., v. 8, p. 385–399, 7 fig.
——— and EMERSON, Sterling H.
1922. GENETIC INTERRELATIONS OF TWO ANDROMONOECIOUS TYPES OF MAIZE, DWARF AND ANTHER EAR. *In* Genetics, v. 7, p. 203–236, 6 fig. Literature cited, p. 226.
HALDANE, J. B. S.
1919. THE PROBABLE ERRORS OF CALCULATED LINKAGE VALUES, AND THE MOST ACCURATE METHOD OF DETERMINING GAMETIC FROM CERTAIN ZYGOTIC SERIES. *In* Jour. Genetics, v. 8, p. 291–297.
KEMPTON, J. H.
1920. HERITABLE CHARACTERS OF MAIZE. III. BRACHYTIC CULMS. *In* Jour. Heredity, v. 11, p. 111–115, fig. 10–12.
———
1921. A BRACHYTIC VARIATION IN MAIZE. U. S. Dept. Agr. Bul. 925, 28 p., 8 fig., 19 pl. Literature cited, p. 28.
MONTGOMERY, E. G.
1911. PERFECT FLOWERS IN MAIZE. *In* Pop. Sci. Monthly, v. 79, p. 346–349. 6 fig.

PLATE 1

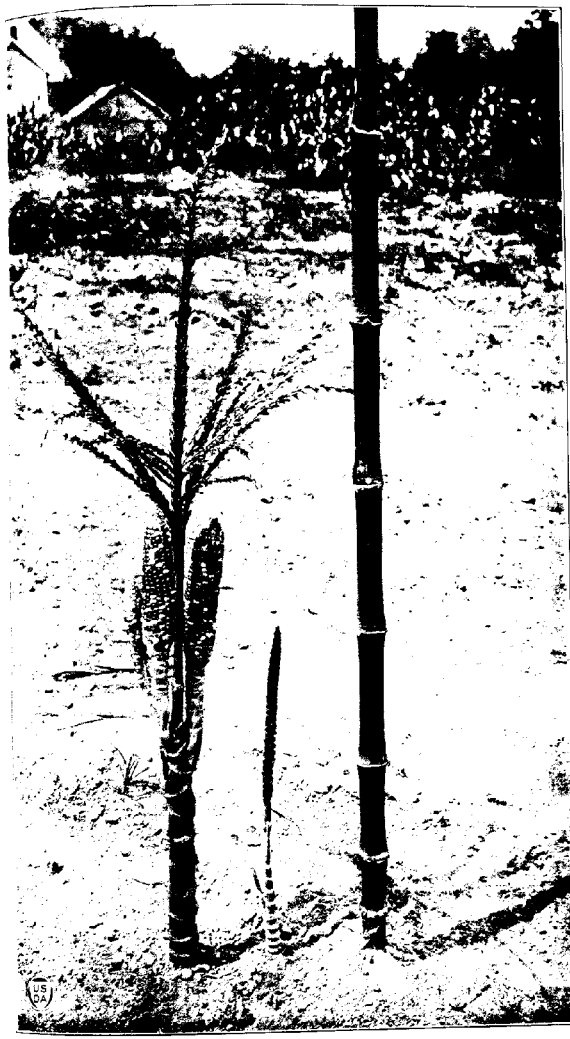
Dwarf and normal maize plants showing relative size. Compare with Plate 2 in which the same normal plant appears photographed at the same scale.

PLATE 2

Normal and brachytic maize plants showing relative heights.

PLATE 3

Brachytic and dwarf maize plants from which the leaves have been removed compared with a section of a plant of normal stature.




A


B

PLATE 4

A.—Dwarf plant from the second generation of the dwarf-brachytic hybrid, showing the type of tassel, sturdy stalk, and short, wide leaves.

B.—Brachytic, dwarf, and brachytic-dwarf maize plants. Brachytic at right, dwarf at left, and the double recessive in center. The brachytic and dwarf plants are sibs from the second generation of the dwarf-brachytic hybrid, while the double recessive plant is from the third generation of this cross, having been obtained in a progeny from a self-pollinated F_2 brachytic plant which proved to be heterozygous for dwarf. No such sharp distinction as is indicated in this picture was found in the second generation between dwarf and the double recessive. It is apparent that the double recessive form of this combination is much larger than the double recessive other ear dwarf figured by the Emersons.

PLATE 5

Three liguleless maize plants from the second generation of the dwarf-brachytic hybrid. At left, a dwarf liguleless; center, brachytic liguleless; right, a plant of normal stature with liguleless leaves. While these plants are from the F_2, they approximate closely the heights of the two parent variations and their F_1 hybrid.

DETERMINATION OF SULPHUR COMPOUNDS IN DRY LIME-SULPHUR[1]

By Carleton Parker Jones

Assistant Research Professor of Chemistry, Massachusetts Agricultural Experiment Station[2]

INTRODUCTION

Lime-sulphur products and analogous substances, known as polysulphids, have of recent years come into prominence because of their value as insecticides and fungicides. Large quantities, especially of the lime-sulphur compound, are now manufactured. Correct and simple methods are needed for the estimation of the principal constituents, both as a safeguard to the producer and as a help to the entomologist and plant pathologist.

METHODS IN USE

Avery (*2*),[3] working with sulphur dips, modified standard methods in order to determine the total sulphur and total lime. Na_2S, resulting from the treatment of a dilute solution with NaOH, was oxidized by a large excess of medicinal H_2O_2.

Haywood (*6*, *7*), also modified standard methods in order to estimate the sulphur combined as sulphids, polysulphids, and thiosulphates. The sulphid sulphur was determined by titration with standard ammoniacal ZnCl, using $NiSO_4$ as an outside indicator. The ZnS was converted into soluble sulphid by treatment with an excess of a saturated solution of KOH. The alkali sulphid was then oxidized by means of a large excess of H_2O_2. The thiosulphate was determined in another portion of the solution, after the removal of the soluble sulphids as explained by titration with standard iodin.

Thompson and Whittier (*9*) determined the monosulphid sulphur by the addition of a slight excess of ammoniacal $CdCl_2$ to the solution of the polysulphid in the presence of KCN. The precipitated CdS was then dissolved in NaOH and the resulting Na_2S was oxidized with H_2O_2. The thiosulphate sulphur was determined in a separate portion by titration of the filtrate, from the ammoniacal $CdCl_2$ precipitation, with standard iodin. The total sulphur was estimated by precipitation with ammoniacal $CdCl_2$ and treatment of the precipitate as given under the monosulphid sulphur determination. The authors state that this method is unaffected by any free $Ca(OH)_2$ or S in solution.

The methods employed by Tartar and Bradley (*8*) consisted in the use of *N/10* ammoniacal $ZnCl_2$ with $NiSO_4$ as an outside indicator, for the determination of the monosulphid sulphur, and a titration method, *N 10* HCl being used with methyl orange as indicator, for the estimation of the polysulphid sulphur. The deposited sulphur was weighed directly, or for more accurate results, recommendation was made to bring the sulphur into solution with KOH and then to oxidize with

[1] Accepted for publication May 2, 1923.
[2] Published with the authorization of the director of the Massachusetts Agricultural Experiment Station.
[3] Reference is made by number (italic) to "Literature cited," p. 336.

H_2O_2. The originators of this method stated that the number of cubic centimeters of HCl used is the amount necessary to react with both the polysulphid and the hydroxid present, the solution always being alkaline due to the hydrolysis of the polysulphid.

Chapin (*3, 4*) originated new methods for the determination, iodometrically, of the three principal forms of sulphur, monosulphid polysulphid, and thiosulphate, the latter being the compound directly titrated in each case. His methods were based, and adjusted accordingly, on solutions containing 1.5 to 2 per cent of sulphid sulphur. Chapin stated that increasing the quantity of sample and using stronger solutions for titrations might very likely improve the accuracy in some of the results.

Harris (*5*) determined both the monosulphid and thiosulphate sulphur in diluted solutions by a double iodin titration. The amount of *N*/10 iodin required to destroy the yellow color of the soluble sulphids was the monosulphid sulphur equivalent. The thiosulphate sulphur was determined by continuing the addition of iodin after the monosulphid end-point had been reached, with starch as indicator. The total sulphur was determined by oxidizing the sulphur in solution with Na_2O_2.

Averitt (*1*) estimated the monosulphid sulphur by titrating a dilute solution with *N*/10 HCl, using in one case either methyl orange or methyl red as an indicator, and in another case sodium nitroprussid as an inside indicator. The thiosulphate sulphur was determined, when either of the first two indicators was used, by titrating with iodin the diluted filtrate, with or without starch, after boiling off the H_2S in solution. When sodium nitroprussid was employed, the thiosulphate sulphur was determined by a further addition of iodin until a faint coloration was produced.

RELATION OF FOREGOING METHODS TO THE CHEMISTRY OF LIME-SULPHUR

When dry lime-sulphur mixtures are subjected to analysis, it is the opinion of the writer that it is entirely unnecessary and erroneous to make a solution of high concentration and then to base quantitative results upon a diluted portion of the highly concentrated solution.

The writer believes that the weak compounds contained in dry lime-sulphur when dissolved in water exist in a complex state of equilibrium so very susceptible even to slight outside influences that these disturbing factors will seriously affect the percentage composition of the constituents if the analysis is based on a diluted solution. It is a well-known fact that polysulphids in aqueous solutions are hydrolyzed to a greater or less degree, depending upon the concentration, and to some extent upon the temperature, of the solution. The hydrolysis of calcium polysulphid might be represented by the following equation:

$$2\ CaS.\ S_x + 2\ H_2O = Ca\ (OH)_2 + Ca\ (SH)_2 + 2\ S_x.$$

The amount of sulphur deposited is of course dependent upon the concentration of the $Ca\ (OH)_2$.

The two points mentioned, (*a*) the action of external factors such as carbon dioxid and oxygen of the atmosphere, and (*b*) the resulting differences in the amount of sulphur in solution due to hydrolysis, should control to a large extent the preparation of the sample and the methods to be applied for the determination of the desired constituents contained in dry lime-sulphur mixtures.

Taking into consideration the above statements, it is believed to be impossible to make accurate comparisons, based on solutions, between different brands of lime-sulphur products when the products so compared are of varying composition. Therefore accurate results can be obtained only by the application of methods directly to the lime-sulphur powder and not to a diluted portion of a highly concentrated solution.

For the determination of thiosulphate sulphur all investigators accept the method used—namely, the titration of the filtrate after the removal of the free sulphur and sulphid, by the use of a standard iodin solution. The procedure followed in obtaining the thiosulphate solution, however, varies, and the results for thiosulphate sulphur are more or less influenced according to the method employed for the separation of polysulphid sulphur from the thiosulphate solution.

The determination of total sulphur is essentially alike in all cases, the total sulphur in solution being oxidized to sulphate either by the use of H_2O_2 or Na_2O_2, and determined as $BaSO_4$.

The important differences between the various methods described are in the determination of the monosulphid and the so-called polysulphid sulphur. The determination of the former by the use of an *N/10* ammoniacal solution of $ZnCl_2$ with $NiSO_4$ as an outside indicator is considered, theoretically at least, as being sound, but the difficulty experienced in obtaining pure zinc and the laborious method of procedure in the use of an outside indicator would make such a method secondary to any other method whose accuracy could be relied upon.

The inaccuracy in the estimation of monosulphid sulphur by standard iodin solution is due largely to the fact that hydrolysis of the calcium polysulphid occurs, and in consequence the iodin reacts also with the base formed, giving too high a value for the monosulphid sulphur.

The same criticisms may be applied to the iodin method as modified by Averitt. Though the sensitiveness of sodium nitroprussid is reduced when it is used with iodin as an inside indicator, this use differentiates an end-point more clearly by the production of color than does the use of iodin alone by the destruction of the color of sulphids in solution.

The end-point for the thiosulphate determination is unsatisfactory, with or without starch, because of the instability in the color developed by the addition of iodin.

The use of *N/2* HCl is preferable to *N/10* HCl for the titration of the sulphid solution, since the end-point with methyl orange is much sharper. The titration with HCl can be so conducted as to result in no appreciable action upon the thiosulphate in solution. The inaccuracy, resulting from calculating the amount of sulphid sulphur present from the number of cubic centimeters of HCl used, is believed to be due to the fact that the amount of HCl required is more nearly a measure of almost the total amount of calcium present in the solution.

The methods in which metallic salt solutions are employed for the determination of the polysulphid sulphur produce colloidal substances and the chemist is confronted with the difficulties of adsorption, slowness of filtering, and possible oxidation of sulphid.

DESCRIPTION OF PROPOSED METHODS

METHOD A

In this method the solutions used were: Hydrated sodium peroxid; barium chlorid solution, 1 to 10; *N/20* iodin; and starch.

The hydrated sodium peroxid is prepared by placing C. P. sodium peroxid under a bell jar with a separate container of water. Air is excluded to prevent the formation of sodium carbonate. The yellow color of the peroxid gradually disappears with the absorption of moisture and the hydrate ($Na_2O_2 8H_2O$) is formed. This hydrated product can be dissolved in water at room temperature with but slight decomposition, or in cool water with practically no decomposition at all. The solution of sodium peroxid may be brought to the temperature of the steam bath with but slight loss of oxygen, and if used at this higher temperature increases the rate of oxidation of the hydrogen sulphid.

PROCEDURE

Sufficient hydrated sodium peroxid and 100 cc. of distilled water are added to flasks No. 1 and No. 2 (fig. 1) and the flasks connected to the apparatus; 0.5 gm. of dry lime-sulphur is transferred to the reaction

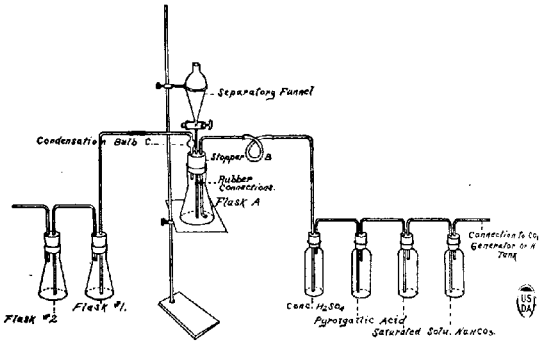


FIG. 1.—Apparatus for the direct separation of the three forms of sulphur—namely, monosulphid, thiosulphate and polysulphid sulphur (plus any free sulphur). The hydrogen sulphid or monosulphid sulphur will be in solution in flask No. 1, thiosulphate sulphur in solution in flask A, and the precipitated or residual sulphur in flask A will consist of the polysulphid sulphur plus any free sulphur.

flask, A. Carbon dioxid (generated by the action of hydrochloric acid upon marble in a Kipp generator), freed from all traces of hydrochloric acid and moisture by passage through a saturated solution of sodium bicarbonate and then through a solution of concentrated sulphuric acid, is forced through the apparatus to replace all air, and without shutting off the current of carbon dioxid, 50 to 60 cc. of distilled water are added to flask A by means of the separatory funnel. The reaction flask is shaken continuously for from three to five minutes, after which time the agitation is carried out intermittently until all the hydrogen sulphid has been expelled and absorbed in the alkaline solutions contained in flasks No. 1 and No. 2.

The contents of flask A are filtered, after the complete expulsion of all traces of hydrogen sulphid, through a platinum Gooch crucible with a thin asbestos pad, and the residue in the crucible is well washed and the filtrate made up to a volume of 200 cc. and titrated with *N*/20 iodin with starch solution as indicator. The iodin titration determines the thiosulphate sulphur.

Any sulphur adhering to the two pieces of glass tubing which dipped into the solution contained in flask A, is added to the bulk of sulphur in the crucible, and the contents of the crucible are treated with 1 to 10 hydrochloric acid to dissolve the precipitated calcium carbonate. The residue is well washed, after which the crucible and contents are dried at 100° C. for one hour, weighed, ignited, and again weighed. The difference in weight represents the sulphur present in the polysulphid given together with any free sulphur.

The sulphur evolved as hydrogen sulphid, and contained in the solution in flasks No. 1 and No. 2[4] as sodium sulphate, is transferred to 500 c. graduated flasks and heated upon the steam bath with the flasks stoppered until complete oxidation is assured. The solution is cooled and a few drops of methyl orange are added, followed by hydrochloric acid until the solution is slightly acid. It is then boiled to destroy the hydrogen peroxid and to drive out all carbon dioxid, cooled, and made to volume.

To 50 cc. portions of the solution ½ cc. of 1 to 1 HCl is added, and the sulphate determination is carried out in the usual manner.

METHOD B

In this method the solutions used are: Hydrated sodium peroxid; approximate *N/2* HCl; barium chlorid solution 1 to 10; *N/20* iodin; and starch.

PROCEDURE

Five-tenths gm. dry lime-sulphur is placed in a small Erlenmeyer flask, 50 to 60 cc. of recently boiled and cooled distilled water added, and the solution titrated, with vigorous agitation of the contents of the flask, with approximate *N/2* HCl, and 3 to 4 drops of methyl orange indicator introduced just previous to the end-point of the reaction. The addition of HCl is continued until the pink color developed persists in the solution after the flask has stood for two or three minutes. The amount of HCl used determines the total basicity of the solution.

The prepared Na_2O_2 solution is then added to flasks No. 1 and No. 2 as in Method A.

Another 0.5 gm. sample of the dry lime-sulphur mixture is transferred to the reaction flask, A, and oxygen-free N passed through the apparatus. To the separatory funnel is added the same quantity of approximate *N/2* HCl which was required to neutralize the basic constituents contained in 0.5 gm. of dry lime-sulphur. The sides of the funnel are rinsed with 50 cc. of distilled water and the diluted acid solution is carefully admitted to the reaction flask without interruption of the slow passage of N. The funnel is again rinsed with a small amount of water, a few drops of methyl orange added, and the solution run carefully into the reaction flask.

The solution contained in flask A should develop a pink color provided all the acid has been removed from the separatory funnel and the separated sulphur has not completely absorbed the indicator.

The passage of N through the apparatus is continued until all traces of H_2S have been expelled from flask A. From this point the procedure is similar to that given under Method A.

[4] Flask No. 2 is used as a precaution and will be found to contain no sulphur, provided the gas is not passed through the apparatus at too rapid a rate.

ADVANTAGES OF PROPOSED METHODS

The advantages derived from the use of either of the two methods are based on accuracy, simplicity of reactions, and the employment of but few and simple operations.

The claim for accuracy is based on the following reasons:

1. Both methods are based directly on the use of dry lime-sulphur and not upon prepared solutions. The methods are applicable, however, to solutions of lime-sulphur.

2. All results are obtained on the one sample throughout the determination.

3. The liberation of sulphur, equivalent to the monosulphid sulphur of the metal, is made complete by the action of dilute acids upon the polysulphid in solution.

4. The evolution of the hydrogen sulphid and the complete removal by aid of a gas allows clear and complete separation of the three forms of sulphur.

5. The determination of monosulphid sulphur, when present in large quantities, can not be more accurately made than by the evolution method; and the absorption, oxidation, and weighing as $BaSO_4$ is as accurate a method for the final determination as any method yet devised.

6. The thiosulphate sulphur is determined by the usual and exact method of oxidation by means of iodin.

7. The sulphur remaining after the complete removal of the monosulphid sulphur is readily separated from the thiosulphate solution by simple filtration and is determined by weight.

The application of these methods is simple because:

1. Complexity in reactions and processes is eliminated by the use of one process for the complete separation of the three forms of sulphur.

2. The time required for the determination is reasonably short, and the process of expelling the hydrogen sulphid from solution does not require the constant attention of the operator.

3. The use of a solution, in connection with hydrogen sulphid, which answers the purpose of an absorbing and oxidizing agent saves time and promotes accuracy.

DISCUSSION OF METHODS

These methods do not include any procedures dealing with the detection or estimation of any of the possible and theoretical sulphur compounds stated by a few investigators as existing in solution as traces, but are concerned wholly with the determination of the principal constituents contained in the commercial lime-sulphur products, which are considered to give to lime-sulphur solutions their insecticidal and fungicidal value—namely, monosulphide and residual and thiosulphate sulphur.

Both methods are based upon the fact that the acids used are ionized to a greater extent than the hydrosulphuric, and therefore have the power of decomposing completely the polysulphids of calcium, with liberation of hydrogen sulphid and deposition of sulphur, according to the following equations:

$$1.\ CaS.S_x + H_2O + CO_2 = CaCO_3 + H_2S + S_x.^5$$

$$2.\ CaS.S_x + 2\ HCl = CaCl_2 + H_2S + S_x.$$

[5] Evidence that carbonic acid completely decomposes the sulphids in solution can be obtained by testing the solution at the end of the experiment with sodium nitroprussid.

The method as represented by the first equation might be designated as the carbonic acid method, and is called method A; the other, as represented by the second equation, might be designated as the hydrochloric-acid method, and is referred to as method B.

No attempt is made by the use of either of the proposed methods to calculate the amount of polysulphid sulphur present in the dry lime-sulphur mixture, because of the uncertainty as to just what proportion of the residual sulphur (contained in the reaction flask) can be attributed to polysulphid sulphur and what amount to soluble free sulphur.

The same uncertainty is apparent upon application of methods based on aqueous solutions of polysulphids. If the lime-sulphur mixture contained free sulphur, the addition of water resulting in the solution of the lime-sulphur compounds would naturally precipitate some if not all of the free sulphur in solution, the quantity deposited depending upon the dilution. On the other hand, the hydrolysis of polysulphids in aqueous solutions results also in the deposition of sulphur, the amount depending upon the dilution and temperature of the solution and the concentration of calcium hydroxid formed. In either case, whether methods are applied to the dry lime-sulphur products or to solutions of the same, it is quite impossible to differentiate accurately between true polysulphid of sulphur and free sulphur in solution.

The true polysulphid sulphur might be estimated with some accuracy, provided there existed a method for determining different amounts of free sulphur in the presence of water soluble polysulphids.

The fact is recognized by the writer that the total amount of soluble sulphur can be estimated only by analysis of the solutions of lime-sulphur mixtures. But in such analyses the solution used should be of the same concentration as that used for spray or as that recommended by the manufacturer of the product.

EXPERIMENTAL TESTS WITH METHODS A AND B

The samples of dry lime-sulphur used in the following experiments were received from the same manufacturers upon two different occasions. The results given in Table I are from a sample received and analyzed in 1919. All other results are from a sample received and analyzed in 1921.

The methods taken for comparison were the HCl and iodin methods recommended by Averitt. They were chosen because of the advantages claimed by the originators and others. There can be no criticism in regard to the quickness and ease of manipulation, but the inaccuracy of results is clearly apparent from the following work.

It was thought that numerous comparisons were unnecessary, provided it could be proved that the use of H_2CO_3 results in a quantitative separation of the monosulphid sulphur from the thiosulphate sulphur and that the use of an excess of CO_2 has no appreciable effect upon the calcium thiosulphate in solution. The experimental work as given in the following pages is believed to substantiate the accuracy of the proposed methods.

TABLE I.—*Comparison between carbonic method and the hydrochloric and iodin method recommended by Averitt for determining soluble sulphur found in sample of dry lime-sulphur*

Form of sulphur determined.	Methods recommended by Averitt.		Carbonic method A.
	Hydrochloric.	Iodin.	
	Per cent.	*Per cent.*	*Per cent*
1. Monosulphid [1]	13. 21	11. 25	8. 3
2. Thiosulphate	1. 56	1. 40	1. 5
3. Residual	50. 84	48. 03	51. 2
Total	65. 61	60. 68	61. 0

[1] The differences in the determination of monosulphid sulphur as shown in this table are very marked, and similar variations were observed when the methods were applied to other samples.

TABLE II.—*Results obtained by use of the carbonic method A*

Experiment No.	Dry lime-sulphur.	Length of time of experiment.	Residual sulphur.	Thio-sulphate sulphur.	Mono-sulphid sulphur.	Absorption solution.
	Gm.	*Hours.*	*Per cent.*	*Per cent.*	*Per cent.*	
1	0. 25	1½	50. 64	2. 75	8. 32	Na_2O_2.
2	. 25	1¾	50. 60	2. 73	8. 35	Do.
3	. 25	1¾	50. 68	2. 75	8. 33	Do.
4	. 25	1¾	50. 64	2. 75	8. 40	Do.
5	. 25	1¾	50. 68	2. 75	8. 34	Do.
Average			50. 65	2. 75	8. 35	
6	. 50	4¼	51. 00	2. 62	8. 41	Do.
7	. 25	1	50. 64	3. 01	(a)	Ammoniacal H_2O_2.
8	. 25	2	50. 64	3. 01	.....	Do.
9	. 50	2	50. 64	2. 56	.....	Do.
10	. 50	2	50. 64	2. 56	.....	Do.
Average			50. 64	2. 79	.....	
11	1. 00	3½	50. 55	2. 58	.....	Do.
12	. 25	17½	50. 76	2. 73	.....	Do.
Average			50. 66	2. 66	.....	

[a] Monosulphid sulphur was not determined for the reason that the H_2O_2 solution used was later found to contain sulphate.

TABLE III.—*Results obtained by use of the carbonic method A*—Continued

Experiment No.	Dry lime-sulphur.	Length of time of experiment.	Residual sulphur.	Thio-sulphate sulphur.	Mono-sulphid sulphur.	Absorption solution.
	Gm.	*Hours.*	*Per cent.*	*Per cent.*	*Per cent.*	
1	0. 5	5	50. 63	2. 64	8. 40	3.6 normal NaOH.
2	. 5	5	50. 64	2. 61	8. 35	4.7 normal NaOH.
3	. 5	5	50. 60	2. 58	8. 35	NH_4OH.
Average			50. 62	2. 61	8. 37	
4	. 5	4	50. 56	2. 68	[a] 7. 91 [b] 8. 46	Weak standard I.
5	. 5	3½	50. 60	3. 00	[a] 8. 33 [b] 8. 32	Weak standard I.

[a] By titration with standard $Na_2S_2O_3$ solution. [b] By weight of precipitated sulphur.

The data given in the Tables II and III show that the results secured for the different forms of sulphur—residual, thiosulphate, and monosulphid—are quite concordant, whether the length of time of the experiment was one hour or a longer period. The use of hydrated Na_2O_2, 3.6 and 4.7 normal NaOH, and NH_4OH also served satisfactorily as absorption solutions. Further experiments with iodin were not as satisfactory.

Further evidence of the completeness of the action of carbonic acid is borne out by a comparison with the results obtained by the use of hydrochloric acid. Five-tenths gm. of dry lime-sulphur from the same sample used in establishing the proposed methods was transferred to the reaction flask, A, dry and oxygen-free CO_2 passed through the apparatus, a sufficient quantity of distilled water added to cover the ends of the inlet tubes, and, finally, 8.36 cc. of *N/2* HCl, an amount previously found necessary to neutralize the basicity of the solution, were slowly added to the sulphid in solution. All traces of acid adhering to the separatory funnel were removed by rinsing with water containing a few drops of methyl orange. That the quantity of acid added was sufficient was indicated by a faint pink coloration of the solution. The contents of the flask were agitated during and after the admission of the acid, and CO_2 was passed through the solution for a period of 4½ hours.

The results obtained upon analysis of the three forms of sulphur are as follows:

Residual sulphur	50.96 per cent.
Thiosulphate sulphur	2.48 per cent.
Monosulphid sulphur	8.47 per cent.

Another experiment was made, using the same weight of dry lime-sulphur from the same sample but increasing the amount of *N/2* HCl to a large excess over that actually required and substituting pure nitrogen gas in place of CO_2. The solution in this case was brought to the boiling point near the end of the operation. The results obtained are as follows:

Residual sulphur	51.56 per cent.
Monosulphid sulphur	8.35 per cent.

The percentage results obtained for monosulphid sulphur in these two experiments are in close agreement with the results obtained by the use of the carbonic-acid method. The slight increase in the percentage of residual sulphur is due to the action of HCl upon the thiosulphate in solution which causes a precipitation of sulphur.

DETERMINATION OF MONOSULPHID AND THIOSULPHATE SULPHUR AFTER A PREVIOUS SEPARATION OF SULPHID FROM THIOSULPHATE

The accuracy of the monosulphid and thiosulphate sulphur results as determined by the carbonic-acid method was again established by the following experimental work in which the same sample of dry lime-sulphur was used.

Five-tenths gm. of dry lime-sulphur was transferred to a small Erlenmeyer flask, to which was quickly added an excess of freshly prepared and well-washed $ZnCO_3$ held in water suspension. The flask was immediately stoppered and shaken vigorously, and the shaking repeated at intervals during a period of one hour, after which the ZnS mixed with free S and excess $ZnCO_3$ was filtered and washed free from the thiosulphate in solution. The thiosulphate sulphur, which was found to equal 2.65 per cent, was determined by titrating the filtrate with *N/20* iodin.

The ZnS mixture upon the filter paper was transferred, by washing, into the reaction flask, A, air was removed from the apparatus by the use of N, HCl in excess added to decompose the carbonate and sulphid, and the gases evolved were expelled by the further use of N. The monosulphid sulphur was determined in the usual way, by oxidation to sulphate and weighing as $BaSO_4$, and was found to be equivalent to 8.4 per cent.

The employment of $ZnCO_3$ in water suspension as a means of separation of the sulphid from the thiosulphate was chosen because of the rapid rate of reaction between the $ZnCO_3$ and the soluble sulphid, the readiness with which filtration could be accomplished, and, furthermore, for the reason that constant results for thiosulphate sulphur were found not to be dependent upon the length of time of contact between the $ZnCO_3$ and the soluble sulphur compounds, as was the case when $CdCO_3$ was used.

ACTION OF CARBONIC ACID UPON CALCIUM POLYSULPHID

The power of carbonic acid to decompose water soluble sulphids can be easily demonstrated by the passage of carbon dioxid into an aqueous solution of lime-sulphur compounds. The completeness of the reaction can be positively verified by testing the solution in which the sulphid was dissolved by the introduction of sodium nitroprussid, after the carbonic acid has been allowed to react for a sufficient length of time.

The time necessary for the complete decomposition of polysulphid is very short, as indicated by the disappearance of the yellow color of the polysulphid in solution. Complete decomposition was effected, repeatedly, within from two to three minutes, when 0.25 gm. charges were used. In the case of larger charges the time was naturally lengthened, requiring from three to four minutes with charges as large as 0.5 gm. The time is based upon the passage of carbon dioxid at a moderate rate. In Table II are tabulated results expressing the quantitative reaction between carbonic acid and lime-sulphur compounds.

EFFECT OF CARBONIC ACID UPON THIOSULPHATE

Investigators of lime-sulphur solutions who have made use of HCl in the process of analysis claim that the neutralization of the solution can be brought about by the careful addition of *N/20* acid, in the presence of methyl orange or methyl red as indicator, without its reacting upon the thiosulphate. If a highly ionized acid, such as HCl, can be so used without destroying any of the thiosulphate, then the chances of a destructive action occurring with the use of an acid which is ionized to a much less degree should be, theoretically at least, even less.

In corroboration of this reasoning, experiments were conducted for the purpose of ascertaining whether or not the action of carbonic acid for different lengths of time had any appreciable effect upon the thiosulphate. The results given in Table II indicate that the weakly ionized acid does not react with the thiosulphate. The occurrence of the observed slight differences in the amounts of thiosulphate sulphur may be due to slight variations in the homogeneity of the lime-sulphur mixtures, or to slight changes in the chemical constituents resulting from repeated openings of the bottle containing the lime-sulphur powder. The perfect agreement in results between a one-plus hour, and the 17½-hour period might be assumed as conclusive proof that the concentration of hydrogen-ions resulting from the solution of H_2CO_3 is without effect upon a solution of calcium thiosulphate.

HYDROCHLORIC ACID AS A MEASURE OF THE BASICITY OF THE SULPHID SOLUTION

That the number of cubic centimeters of HCl used in the direct acid titration method can not be taken as an index of the quantity of monosulphid sulphur present in lime-sulphur mixtures, but may be taken as a measure of the basicity of the solution, is borne out by the following experiments.

Five-tenths gm. charge of dry lime-sulphur was analyzed by the carbonic acid method; CO_2 was passed through the solution for four hours, the solution brought to boiling, and the CO_2 passed through the solution for another hour. The contents of the reaction flask, A, containing free S, $CaCO_3$, $Ca(HCO_3)_2$, and CaS_2O_3, were then titrated with *N/2* HCl, using methyl orange as indicator. It was found that 8.23 cc. of acid were required to neutralize the solution.

Upon application of the direct acid titration method (Averitt) it was found that 8.26 cc. of *N/2* HCl were necessary to carry the reaction to the end point of methyl orange. The number of cubic centimeters of acid used according to the method is the equivalent of the monosulphid sulphur present. Therefore the amount of the latter, based on a 0.5 gm. sample, should equal 13.24 per cent.

A comparison of these two simple tests shows that in one case 8.26 cc. of *N/2* HCl were used in neutralizing the polysulphid solution, while in the other case almost an equal amount of HCl was required to neutralize the basic salts present, after the removal of the monosulphid sulphur.

The only possible deduction is that the HCl reacts with not only the calcium sulphid but other calcium compounds as well, and consequently the amount of HCl required can not be used as a direct estimation of the monosulphid sulphur present.

That 13.24 per cent for monosulphid sulphur is much too high is again made apparent by completing the former experiment in the usual way. The results obtained for the three forms of sulphur are as follows:

Residual sulphur	50.64 per cent.
Thiosulphate sulphur	2.61 per cent.
Monosulphid sulphur	8.35 per cent.

ABSORPTION SOLUTIONS

Different absorptive solutions for the evolved H_2S were substituted in place of the hydrated sodium peroxid. Among the solutions tried were the following: Ammoniacal H_2O_2, ammoniacal $ZnCl_2$, ammoniacal $CdCl_2$, acetic acid solution of $Zn(C_2H_3O_2)_2$, dilute standard solution of iodin with a protective flask containing a standard solution of $Na_2S_2O_3$), 3 to 4 *N* KOH and NaOH solutions.

With the exception of the alkali solutions, all these proved unsatisfactory for one reason or another. Solutions of hydrogen peroxid invariably contain sulphates in greater or less quantity and consequently necessitate the employment of exact measured amounts and require that blank tests be made upon each lot of peroxid solution used. The use of zinc and cadmium solutions, neutral or alkaline, gives precipitates of metallic sulphids which are of a decidedly colloid nature. The difficulties attending filtration and washing are well known. Oxidation of the sulphids during the operation is a constant source of error tending toward low results. The sulphid can not be weighed directly with accuracy because of occlusion, and, therefore, must necessarily be changed into some other form in order that the estimation of the sulphur content may be made.

Iodin solutions used as an absorbent in the determination of the monosulphid sulphur must be very dilute as the tendency for the precipitated sulphur to oxidize increases with increase in concentration. Occlusion of iodin by the sulphur and the volatilization of iodin, due to the passage of the gas through the solution, were found also to increase with increased concentration of the iodin solution. Good results were obtained, however, in a few determinations (Table III) by the use of a weak iodin solution *N/20* or less, containing an appreciable quantity of acetic acid and a large amount of potassium iodid. Starch solution in the train following the iodin solution showed no volatilization of iodin. The determination of sulphur by weighing was in agreement with the estimation made by titrating the excess of iodin. On the average the use of an iodin solution as a means of determining the monosulphid sulphur was found unreliable.

There is no gain in accuracy in the use of pure alkali solutions as absorptive agents over that obtained by the employment of a hydrated sodium peroxid solution (Tables II and III). The operation is somewhat lengthened for the reason that after the absorption the sulphur must be oxidized. The oxidation is best accomplished by the use of sodium peroxid which can easily be obtained free from sulphates.

In the opinion of the writer it is advantageous, as well as accurate, to make use of a solution which answers the purpose of an absorptive and oxidizing solution at the same time.

RESULTS FOR TOTAL SULPHUR

COMPARISON BETWEEN THE CARBONIC ACID METHOD AND METHOD OF DIRECT OXIDATION

The total sulphur was determined by direct oxidation with Na_2O_2. To 0.25 or 0.5 gm. of dry lime-sulphur were added 50 to 100 cc. of freshly boiled and cooled distilled water. Hydrated Na_2O_2, or the dehydrated form, was then added in small amounts until the oxidation was completed, the flask being kept stoppered. The procedure which followed was the same as that used in determining the monosulphid sulphur. A comparison of the total sulphur determined in this way with that obtained by the summation of the three forms as determined by the carbonic acid method (average of results given in Tables II and III) is given below.

		Total sulphur
Direct oxidation		61.62 per cent.
H_2CO_3 method	Residual sulphur	50.64 per cent.
	Thiosulphate sulphur	2.70 per cent.
	Monosulphid sulphur	8.36 per cent.
		61.70 per cent.

HYDROLYTIC ACTION RESULTING FROM AQUEOUS SOLUTIONS OF LIME-SULPHUR COMPOUNDS.

Figure 2 is illustrative of the hydrolysis of lime-sulphur compounds in aqueous solutions and is therefore indicative of errors which may arise when the analysis of dry lime-sulphur is based on diluted portions of concentrated aqueous solutions.

The experiments were conducted as follows:

The different amounts of dry lime-sulphur were taken and transferred to a 500 cc. Erlenmeyer flask [6] and freshly boiled distilled water cooled

[6] The same flask was used for each experiment.

to 20° C. was added until a volume of 535 cc. resulted. The flask was immediately corked and sealed with paraffin, leaving but a very small air space. The flask was then allowed to stand at room temperature with shaking for 48 hours, after which the deposited sulphur was rapidly transferred, by forced filtration, to a platinum Gooch crucible with a thin asbestos pad, thoroughly washed, dried for one hour at 100° C., and the crucible and contents weighed, ignited, and reweighed to obtain the amount of sulphur.

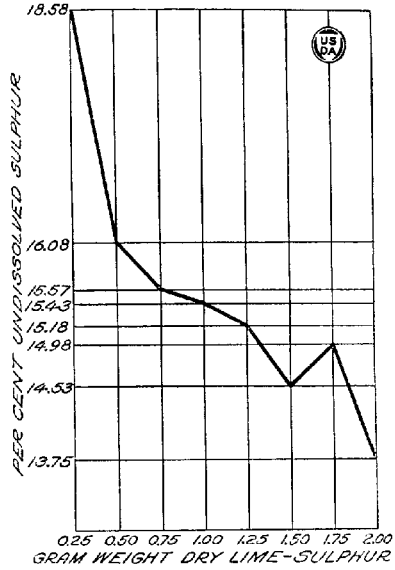


FIG. 1.—Diagram showing variations in percentage of undissolved sulphur due to chemical equilibrium resulting from the hydrolysis of lime-sulphur compounds in aqueous solutions.

SUMMARY

(1) The three forms of sulphur, monosulphid, residual, and thiosulphate sulphur, contained in lime-sulphur powders may be easily and accurately determined by the passage of CO_2 through a solution of the polysulphid. The accuracy of the method is based upon the fact that CO_2 separates quantitatively the monosulphid sulphur from the thiosulphate and residual sulphur. The use of this method allows for a complete separation of the residual from the thiosulphate sulphur, thus enabling accurate estimation of both.

(2) The method is entirely free from any laborious process of filtering and uncertainty regarding end-points in the use of indicators.

(3) The application of this method eliminates all errors due to hydrolysis of lime-sulphur compounds in aqueous solutions and also the reacting influences of CO_2 and O of the atmosphere.

(4) The use of hydrated Na_2O_2 for the absorption of H_2S has a twofold advantage. The alkalinity of the solution acts as a binding agent while the 20 per cent of available O acts in the capacity of a powerful oxidizer.

LITERATURE CITED

(1) AVERITT, S. D.
1916. SEPARATION AND ESTIMATION OF POLYSULFIDES AND THIOSULFATE IN LIME-SULFUR SOLUTIONS. *In* Jour. Indus. and Engin. Chem., v. 8, p. 623–627. Bibliographical footnotes.

(2) AVERY, S.
1905. THE DETERMINATION OF LIME AND SULPHUR IN SOLUTIONS OF SULPHIDS OR POLYSULPHIDS USED AS INSECTICIDES. *In* U. S. Dept. Agr. Bur. Chem. Bul. 90, p. 104–105.

(3) CHAPIN, Robert M.
1916. SOME NEW METHODS FOR THE ANALYSIS OF LIME-SULFUR SOLUTIONS. I. Jour. Indus. and Engin. Chem., v. 8, p. 151–156. Bibliographical footnotes.

(4) ———
1916. NEW METHODS FOR THE ANALYSIS OF LIME-SULFUR SOLUTIONS. II. THE ESTIMATION OF "POLY-SULFUR." *In* Jour. Indus. and Engin. Chem., v. 8, p. 339–341. Bibliographical footnotes.

(5) HARRIS, James E.
1911. LIME-SULFUR SPRAY. Mich. Agr. Exp. Sta. Tech. Bul. 6, 15 p.

(6) HAYWOOD, J. K.
1905. COMPOSITION OF THE LIME, SULPHUR AND SALT WASH. *In* Jour. Amer. Chem. Soc., v. 27, p. 244–255.

(7) ———
1907. THE LIME-SULPHUR-SALT WASH AND ITS SUBSTITUTES. U. S. Dept. Agr. Bur. Chem. Bul. 101, 29 p.

(8) TARTAR, H. V., and BRADLEY, C. E.
1910. ON THE COMPOSITION OF LIME-SULFUR SPRAY. *In* Jour. Indus. and Engin. Chem., v. 2, p. 271–277. Bibliographical footnotes.

(9) THOMPSON, Firman, and WHITTIER, A. C.
1914. THE COMPOSITION OF LIME-SULFUR SOLUTIONS. Del. Agr. Exp. Sta. Bul. 105, 30 p.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT
10 CENTS PER COPY
SUBSCRIPTION PRICE, $4.00 PER YEAR